Diagnosis and Management of Sore Throat in Young Adults

AUTHOR(S)

Muireann De Paor

CITATION

De Paor, Muireann (2022): Diagnosis and Management of Sore Throat in Young Adults. Royal College of Surgeons in Ireland. Thesis. https://doi.org/10.25419/rcsi.19755436.v1

DOI

10.25419/rcsi.19755436.v1

URL

https://repository.rcsi.com/articles/thesis/Diagnosis_and_Management_of_Sore_Throat_in_Young_Adults/19755436/1

UNIVERSITY OF MEDICINE AND HEALTH SCIENCES
RCSI

Diagnosis and management of sore throat in young adults

A thesis submitted to the Royal College of Surgeons in Ireland for the degree of Doctor of Philosophy in the Department of General Practice

Dr Muireann de Paor
MB BCh BAO, BMedSc, DOWH, DMSM, MICGP, DHPE

Supervisors

Professor Tom Fahey
Professor Susan Smith
Dr Fiona Boland

February 2022

Candidate Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree of Doctor of Philosophy, is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own.

I have not already obtained a degree in RCSI or elsewhere on the basis of this work.

Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

Signed 

Student Number 15134440

Date 9th May 2022

Table of Contents

List of Abbreviations

AAFP	American Academy of Family Physicians
AAP	American Academy of Pediatrics
ABRS	Acute bacterial rhinosinusitis
ACP-ASIM	American College of Physicians–American Society of Internal Medicine
ACS	Acute coronary syndrome
ACV	Acyclovir
ADNaseB	antideoxyribonuclease B
AHA	American Heart Association
ARI	Acute respiratory infections
ARS	Acute rhinosinusitis
ASOT	Antistreptolysin O titre
AUC	Area under the curve
BMI	Body mass index
CAP	Community acquired pneumonia
CDC	Center for Disease Control
CDR	Clinical decision rule
CDSS	Clinical decision support system
CENTRAL	Cochrane Central Register of Controlled Trials
CFS	Chronic fatigue syndrome
CI	Confidence interval
CINAHL	Cumulative Index to Nursing and Allied Health Literature
CIT	Cork Institute of Technology
CMV	Cytomegalovirus
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CPR	Clinical prediction rule
CRF	Case report form
CRP	C-reactive protein
DIT	Dublin Institute of Technology
DNA	Deoxyribonucleic acid

DOR	Diagnostic odds ratio
EBNA	Epstein Barr virus nuclear antigen
EBV	Epstein Barr Virus
EMA	European Medicines Agency
EMBASE	Excerpta Medica database
ESCMID	European Society for Clinical Microbiology and Infectious Diseases
FBC	Full blood count
FSS	Fatigue severity scale
GABHS	Group A Beta Haemolytic Streptococcus
GP	General Practitioner
GRADE	Grading of recommendations, assessment, development and evaluations
HHV-6	Human Herpesvirus-6
HIV	Human immunodeficiency virus
HSE	Health Service Executive
HSV	Herpes simplex virus
ICSI	Institute for Clinical Symptom Improvement
ICTRP	International Clinical Trials Registry Platform
IGM	Immunoglobulin M
ILI	Influenza-like illness
IM	Infectious mononucleosis
IQR	Interquartile range
IRCT	Iranian Registry of Clinical Trials
ISDA	Infectious Diseases Society of America
IT	Information Technology
IV	Intravenous
LILACS	Literatura Latino-Americana e do Caribe em Ciencias da Saude
LR	Likelihood ratio
LR-	Negative likelihood ratio
LR+	Positive likelihood ratio
LRTI	Lower respiratory tract infection
MD	Mean difference
MEDLINE	Medical Literature Analysis and Retrieval System Online

MS	Multiple sclerosis
MU	Maynooth University
NICE	National Institute for Health and Care Excellence
NUIG	National University of Ireland Galway
OR	Odds ratio
POCT	Point of care test
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCSI	Royal College of Surgeons in Ireland
RCT	Randomised controlled trial
ROC	Receiver operating characteristics
RR	Risk ratio
SIGN	Scottish Intercollegiate Guidelines Network
SMD	Standardised mean difference
SOB	Shortness of breath
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TCD	Trinity College Dublin
UCC	University College Cork
UK	United Kingdom
UL	University of Limerick
URTI	Upper respiratory tract infection
USA	United States of America
VAS	Visual analogue scale
VCA	Viral capsid antigen
VZV	Varicella Zoster virus
WBC	White blood cell
WCC	White blood cell count
WHO	World Health Organisation

List of Tables

List of Figures

SUMMARY

Background

Sore throat is one of the most common presentations in primary care, especially in young adults. This thesis aimed to further research in the area of improving the diagnosis and management of sore throat in young adults, with a particular focus on infectious mononucleosis (IM) and Group A Beta Haemolytic Streptococcus (GABHS). There were four broad objectives; 1) to systematically review the effectiveness of antiviral therapy for infectious mononucleosis (IM), 2) to derive and externally validate a clinical prediction rule (CPR) to aid in the positive diagnosis of IM caused by Epstein Barr Virus (EBV), 3) to validate the Centor score CPR (a widely used CPR for the diagnosis of GABHS sore throat) in an Irish setting, and 4) to explore potential associations between IM caused by EBV and fatigue and time missed from college / work in younger adults.

Methods

A systematic review of the effectiveness of antivirals for IM was carried out, according to Cochrane methodology. A prospective cohort study with six-month follow-up was conducted in seven student health centres in Ireland. A CPR for the diagnosis of EBV was developed and internally validated in the thesis cohort and externally validated in a geographically separate cohort. The Centor score CPR was validated in this research cohort. The cohort study data was also used to explore outcomes (fatigue scores and time missed from college or work) of participants diagnosed with EBV IM versus those with sore throat from other aetiology.

Results

The systematic review included seven randomised controlled trials (RCTs) with a total of 333 participants. There were small statistically significant improvements in the treatment group for two of the twelve outcomes; 'time to clinical recovery as assessed by physician' and 'duration of lymphadenopathy, however these may not be clinically meaningful. A total of 348 young adults (42 (12.8%) EBV positive) were recruited to the cohort study. Response rates at three month follow up were 68.7% and at six month follow up were 56.3%, with similar rates in EBV positive and EBV negative participants. Four CPR models were

developed for the diagnosis of EBV. The models performed reasonably well in internal validation and similarly when validated externally. When the Centor score was validated in this study population, it was found to have only fair performance. The cohort study was underpowered, but a large percentage of those in both EBV and non-EBV sore throat groups (56% and 42% respectively) reported fatigue six months following acute illness. The odds ratio for missing 10 or more days (compared to 0-9 days) for EBV positive versus EBV negative patients was 3.58 (95% CI 1.47 to 8.71).

Conclusions

The presentation of sore throat is a very common one in primary care. This thesis adds to the literature in the area by examining its diagnosis and management in young adults, especially focussing on two conditions; IM caused by EBV and GABHS sore throat. This thesis describes tools that can aid in the diagnostic process, suggests strategies for management, depending on which underlying pathology is suspected and highlights evidence gaps in the management of IM.

Acknowledgements

First and foremost, thank you to the study participants and student health centres who gave so generously of their time in taking part in this study.

Thank you to the Health Research Board of Ireland (HRB) who funded my Cochrane review and to the HSE who funded an academic fellowship, which allowed me protected time to pursue further PhD research.

I have been very fortunate to receive excellent supervision and mentorship throughout my PhD. Huge thanks is due to Professor Tom Fahey, Professor Susan Smith and Dr Fiona Boland for their direction, reassurance and support, and methodological expertise. Thank you to my colleagues at RCSI who are a great source of inspiration, friendship and encouragement.

Thank you to Dr Brendan Crowley who provided invaluable advice from a microbiological perspective.

Finally, thank you to my siblings, parents, husband and children for their support, encouragement, understanding, and technical expertise throughout.

1 Chapter 1: Introduction

This thesis describes research performed to aid in the diagnosis and management of sore throat in young adults, focussing here on two conditions; infectious mononucleosis (IM) (primarily caused by Epstein Barr Virus-EBV), but also sore throat caused by Group A Beta Haemolytic Streptococcus (GABHS). Ideally the thesis research would have examined IM caused by all pathogens, but for pragmatic reasons only cases of IM caused by EBV could be examined.

The first chapter presents a brief overview of sore throat, with more detail on the condition of infectious mononucleosis (IM), its principal clinical features and its differential diagnoses (principally GABHS). There is a subsequent discussion on tools that can aid in the diagnostic process, the clinician's approach to diagnosis, and, finally, the main study aims are stated.

1.1 Sore throat

Sore throat is a common presenting symptom in primary care, especially in young adults. A systematic review from 2019, including data from primary care patients across twelve countries, found that sore throat was the fourth most common reason for presentation.(1) It is a symptom that disproportionately affects children and younger adults, with incidence declining from the age of forty onwards.(2)

Sore throat can be caused by a number of pathogens; viral (50-80% of cases), bacterial (10-20%), or fungal (<1% of cases), and also from non-infectious causes e.g., gastroesophageal reflux disease or allergic rhinitis.(3-5) It is estimated that in young adults presenting with sore throat, approximately 8% will have EBV as the causative factor, and approximately 15% will have GABHS as the causative factor.(3, 6, 7) The patient's history can help differentiate infectious from non-infectious causes, and the patient's age is an important aspect of this. However, the causative pathogens of cases of acute sore throat can be difficult to distinguish clinically. Viral causes (e.g. rhinovirus, adenovirus, coronavirus, EBV) can often be associated with other features of an upper respiratory tract infection (URTI) e.g. cough,

coryza and fatigue.(8) Symptoms and signs that are characteristically associated with GABHS include presence of tonsillar exudate, pharyngeal exudate and exposure to GABHS infection in the previous two weeks.(9) The absence of tender anterior cervical nodes, tonsillar exudate, or enlargement makes the diagnosis of GABHS sore throat less likely.

Many countries have produced their own guidelines for the diagnosis and management of sore throat, most focussing particularly on the aspect of GABHS and antibiotics. These are discussed in more detail in Section 1.3.3.3.

1.2 Clinical condition: infectious mononucleosis (IM)

1.2.1 Background

Infectious mononucleosis is a clinical syndrome that classically presents with the triad of sore throat, lymphadenopathy and fever.(10) The vast majority of cases of IM in developed countries are caused by the Epstein-Barr virus (EBV), and for many clinicians the terms EBV and IM are synonymous. However, the pathogens cytomegalovirus (CMV), human immunodeficiency virus (HIV), adenovirus, Streptococcus pyogenes, human herpesvirus 6 (HHV-6), Toxoplasma gondii and herpes simplex virus (HSV) account for approximately 10% of cases in developed countries, and occur in different proportions depending on the age, geographical and social context.(10)

EBV is a common herpes virus that is predominantly transmitted through infected saliva. The majority of cases of primary EBV infection during childhood are subclinical, with less than ten percent exhibiting clinical symptoms, however, the incidence of clinical infection rises with age.(11)

1.2.2 Prevalence of EBV infection

Cross sectional studies indicate that approximately 90% of people in the world have been infected with EBV by the end of adolescence.(12) Prevalence in children varies according to geographical location, with infection being acquired later in developed regions.(13, 14) This

has implications for higher numbers in these countries being affected by the syndrome of infectious mononucleosis in young adulthood. A recent sero-epidemiological study carried out in the UK found that the incidence of IM requiring hospitalisation has increased.(15) This study also established that increased risk of IM was associated with white ethnicity, lower BMI and not smoking. Rates of infection are also determined by socioeconomic status. A UK study from 2017 found that material deprivation in early life along with overcrowded living situations were associated with a higher risk of EBV infection among children.(16)

However, it has been found that the incidence of clinical IM is higher in first degree siblings and same sex twins, compared to more distant relatives, so genetic factors may also play a role in the development of the clinical IM syndrome.(17)

A seasonal pattern was observed in one prospective cohort study (of 590 participants), with peak incidence occurring during the summer months, however a winter peak was found in another large retrospective observational cohort study (> 60,000 participants) set in Scotland.(18, 19) Most of the literature in this area has not demonstrated seasonality associated with IM.(20-22)

1.2.3 Transmission and pathogenesis

Following clinical infection, EBV can be shed in saliva at high levels for up to six months, and can be found in the oropharynx intermittently for decades.(23, 24) Transmission occurs mainly through infected saliva, via kissing, coughing, sharing food or utensils, and poor hand hygiene.(25)

The incubation period of IM ranges from four to eight weeks with symptoms peaking one week from onset, and resolving (in most cases) over the following 1-4 weeks.(26, 27) However, EBV integrates itself into the hosts B lymphocytes and remains as a lifelong latent infection which is reactivated periodically.(28) Latency of the virus is characterized by three different processes including persistence of the viral genome, restricted viral gene product expression, and the possibility to reactivate to replication. (29, 30)

1.2.4 Clinical features

EBV infection may be subclinical during childhood years, without causing the overt syndrome of IM. However, the incidence of symptomatic infection rises in adolescents and adults. IM is a clinical syndrome that is characterised by lymphadenopathy, fever and sore throat.(10) There is often a prodrome of headache, low grade fever and malaise before more specific symptoms develop. Splenomegaly is seen in approximately 50% of patients with IM but usually begins to resolve by the third week of the illness.(31)

Acute symptoms usually last for one to two weeks and generally resolve, although reduced functional status and fatigue can continue in some cases for months.(32, 33)

1.2.4.1 Severity scale

There have been scales used to describe the severity of IM in various studies; however, these are heterogenous, particular to each study, and most of them are unvalidated.(23, 34, 35) A recent article described how a validated scale was developed to assess the severity of acute IM.(36) The authors developed their scale, based on a review of the literature in this area, and participants were assigned scores based on the presence of certain severe symptoms, signs or complications. Records of the acute care visit were reviewed by two independent study physicians and the scale was shown to have good interobserver reliability. There was a significant association between severity of the infectious mononucleosis (based on the scale) and hospitalisation, which demonstrated that the scale had validity within its derivation cohort. The patients with a higher severity scale also had a higher risk of developing chronic figure syndrome (CFS) following an acute episode of IM. The scale has not, however, been validated in an external population.

1.2.5 Diagnosis

IM is usually diagnosed clinically, when a patient presents with sore throat and other characteristic signs and symptoms, typically fever, fatigue and lymphadenopathy. Blood testing during the acute phase usually reveals atypical lymphocytosis and abnormal liver function tests (LFTs).(37) Although there are no definitive clinical diagnostic criteria, specific blood tests may be required to confirm diagnosis or to identify the cause of illness in atypical cases. A definitive positive diagnosis aids in the management of patients by avoidance of unnecessary antibiotics, and enabling more accurate advice about prognosis and awareness of potential complications (which on rare occasions, can be life threatening).

Full blood count (FBC) usually reveals a lymphocytosis, with a high proportion of atypical lymphocytes. The Hoagland criteria, developed in 1975, advise that in patients with clinical features of IM and at least 50% lymphocytosis (10% atypical), diagnostic testing should be undertaken.(38) An Irish retrospective analysis of 1000 Monospot tests (500 positive and 500 negative, from both outpatients and in-patients) found that although raised absolute lymphocyte count is a good screening tool, it is not sufficient on its own to diagnose or rule out IM.(39) A recent systematic review and meta-analysis of this and similar studies examined the effect of raised absolute lymphocyte count, ratio of lymphocytes to total white blood cell (WBC) count and proportion of atypical lymphocytes on the diagnostic probability of IM.(40) The authors concluded that atypical lymphocytosis is useful when combined with a high percentage of lymphocytes, e.g., a patient with > 10% atypical lymphocytes and >50% lymphocytes had a positive LR of 50.4,and a specificity of 0.99, however a modest sensitivity of 0.45. FBC is available as a POCT in some countries, however it is not readily available as a POCT in Ireland.

In the past, reactive heterophile IgM antibody testing (commonly known as a 'Monospot test') was considered the reference standard diagnostic test for IM. This is still used in some regions, sometimes as a point of care test, but also as a lab-based test.(41) The Monospot test is very specific; specificity and sensitivity of heterophile IgM antibody testing are approximately 90% and 86% respectively.(42) However, its main drawbacks are that up to 40% of children under the age of four do not develop heterophile antibodies following acute EBV infection, the test can vary during the course of infection and can be falsely negative in approximately 25% of adults in the first week of having symptoms.(10, 38, 42). In addition,

almost 10% of adults with IM will have persistently negative heterophile antibody testing.(43).

A definitive diagnosis can be made by laboratory testing for IgG and IgM antibodies against viral capsid antigens, early antigens and EBV nuclear antigen proteins.(44) This is discussed further in Section 3.2.5. Table 1-1 demonstrates the difference in diagnostic accuracy between the test methods.

Table 1-1: comparison of FBC, heterophile antibody testing and EBV antibody testing

Test	Sensitivity (ref)	Specificity (ref)	Turnaround Time
Lymphocytosis (≥4 × 109 /L lymphocytes)	59% (40)	94% (40)	POCT: 15 mins Lab: 1 day
Lymphocytes >50% + ≥10% atypical lymphocytes	45% (40)	99% (40)	POCT: 15 mins Lab: 1 day
Heterophile antibody testing	86% (38% in <12-year-olds) (42)	90% (100% in < 12-year-olds) (42)	POCT: 15 mins Lab: 1 day
EBV antibody testing	95-100% (45)	86-100% (45)	Lab only; 3 days

Currently, the primary care testing approach varies depending on geographical location and the availability of POCT. In Irish primary care, including student health centres, usually a blood test is taken, to be sent to the local laboratory for EBV serology or a Monospot testing. This is considered further in the discussion section of Chapter 4 (Section 4.5).

1.2.6 Differential diagnosis

Patients presenting with the classical clinical triad of fever, sore throat and lymphadenopathy may have IM caused by a pathogen other than EBV, e.g. CMV, *Toxoplasma gondii,* or HIV.(46) However, one of the most common differentials of IM seen in primary care in Ireland is sore throat caused by GABHS. Differentiating between IM or GABHS sore throat can be difficult clinically, but clinical prediction rules can aid clinicians in deciding which patients need further testing and which patients may be treated empirically.

Other less frequent bacterial causes of sore throat such as Group C / G Streptococcus and Fusobacterium necrophorum have similar symptoms to those of GABHS and can also occasionally cause serious complications. See Table 1-2 for differential diagnoses of IM and their key distinguishing features.

Table 1-2: Infectious mononucleosis: differential diagnosis (45)

Diagnosis	Key Distinguishing Features
Acute human immunodeficiency virus infection	Mucocutaneous lesions, rash, diarrhoea, weight loss, nausea, vomiting
Cytomegalovirus infection	Similar clinical features. Paired IgG serology demonstrates a fourfold increase in antibody titres and a significant elevation in IgM (at least 30% of IgG value)
GABHS sore throat	Absence of splenomegaly or hepatomegaly; fatigue is less prominent
Toxoplasmosis	Recent history of eating undercooked meat or cleaning a cat's litter box
Other viral sore throat	Lymphadenopathy, tonsillar exudates, fever, and absence of cough are less likely than with GABHS sore throat or infectious mononucleosis

IgG = immunoglobulin G; IgM = immunoglobulin M.

1.2.7 Complications

Symptoms of IM usually resolve within the first month, but they may be prolonged, especially fatigue, persisting in some cases up to six months post acute infection.(27) Haematological and hepatic complications can occur but are generally minor and self-limiting; however, rare serious complications such as airway obstruction occur in a small minority ($\leq 1\%$) of patients.(44) A systematic review of 85 case reports of splenic rupture concluded that there is a very small but potentially fatal risk of splenic rupture in approximately 0.1% of cases up to eight weeks after the onset of acute symptoms.(47) In the systematic review, the average patient age was found to be 22 years, while 70% of those affected were male. A preceding history of trauma was only reported in 14% of patients and

overall mortality from splenic rupture was 9%. The authors recommended avoidance of high-risk activities (sports, vigorous activity and heavy lifting) for eight weeks after acute IM.

1.2.7.1 Hospital admission

In general, rates of hospitalisation for IM are low.(48) Recently however, there has been an increase in hospital admissions for IM, with one UK study reporting a coupling of increasing hospital admissions alongside a reduction in GP consultations.(49, 50) An Irish retrospective study examined hospital stays for IM in patients aged over 15, over a 20-year period in a single institution from 1990-2009.(51) The study included 3435 cases in total; 371 with IM and 3064 with bacterial tonsillitis, and found that hospital admission rates for IM in the general population increased from 1.6/100,000 in 1990 to 5.5/100,000 in 2009. The study also found that patients admitted to hospital with IM were significantly younger than patients admitted to hospital with bacterial tonsillitis (means of 20.0 years and 25.7 years respectively), and that patients with IM had a significantly longer hospital stay than those with bacterial tonsillitis (means of 4.37 days and 3.22 days respectively). The authors commented on its limitations; that the study did not include readmissions, and that treatment regimens may also have changed over the twenty-year study period.

1.2.8 Management of IM

As IM is generally self-limiting, management usually consists of supportive care. Adequate fluid and nutritional intake are recommended, along with simple analgesia and antipyretics to relieve symptoms.(44)

There is a lack of prospective studies on athletes returning to play following infectious mononucleosis; however, a 2016 systematic review of available case reports regarding splenic rupture (mentioned above) suggests that athletes should be advised that they should avoid high risk activities for eight weeks following illness onset.(47)

The effectiveness of antiviral agents (acyclovir, valomaciclovir and valacyclovir) in acute IM is uncertain. The previous meta-analysis examining acyclovir for the treatment of IM showed some benefit in reducing oropharyngeal EBV shedding but no evidence to support its clinical effectiveness.(52) This research is outdated and only included one antiviral agent, acyclovir. Thus, a Cochrane review of this question was undertaken in 2016 by the PhD candidate to search, appraise and summarise the available evidence of antiviral agents for IM. The search for this systematic review was updated in December 2021 (See Chapter 2).

A 2015 Cochrane review of steroids for IM did not find sufficient evidence to recommend their use.(53) However, a recent cohort study (the main objective of which was to develop a severity scale for IM), found that the more severe the case of IM, the more likely the patient was to have a prescription written for corticosteroids, although this study only reported the correlation, rather than examining the particular types of cases in which steroids were prescribed.(36, 53) Another review article concluded that steroids may be used to treat some complications of IM, especially airway obstruction or autoimmune complications such as thrombocytopenia and anaemia.(54)

An anaerobic antibiotic, metronidazole, has been studied in severe hospitalised cases of patients with IM and has been found to reduce hospital stay.(55) This was a single centre randomised controlled trial (RCT) published in 2014 with 42 patients enrolled in the trial. The duration of hospital stay was significantly less (p 0.032) in the metronidazole treatment group (3.67 days v 4.67days). Previous small studies of metronidazole for IM generally showed improvement with treatment, however all these studies had less than 50 participants, and were published approximately forty years ago.(56-58) One small study published in 1982 did not find any statistically significant difference between treatment and control groups.(59)

If ampicillin or amoxicillin is administered empirically for the treatment of GABHS sore throat (as a clinical differential diagnosis), when the diagnosis is in fact IM, a maculopapular rash frequently occurs. The pathophysiology for this is not well understood, and patients

who develop the rash usually subsequently tolerate penicillin without an adverse reaction.(60)

1.2.9 Prolonged fatigue

Occasionally, fatigue after acute IM can be severe and persistent. Persistent fatigue was present in 12% of cases at six months after illness onset in one prospective study and a systematic review of similar studies found that 9-22% of patients with IM were classified as having Chronic Fatigue Syndrome (CFS) six months after the acute illness.(61-63) Fatigue appears to be more common and more severe in females compared with males.(26)

Fatigue can be determined in a number of ways, and there is no standardised measure. One of the available validated measures is the Fatigue Severity Scale (FSS). A 2009 systematic review examining the performance of fatigue measures concluded that 'there is no ideal instrument' but that the FSS demonstrates good psychometric properties and demonstrates the ability to detect change over time.(64) Another review of measures of fatigue found that the FSS had excellent reliability and validity.(65) It has been validated in populations with chronic fatigue syndrome but not specifically in populations with fatigue post infectious mononucleosis. Likewise, there are different definitions of CFS used by various studies in this area.(62)

See Section 7.1 for further information on CFS and fatigue following IM.

1.2.9.1 Other long-term consequences

EBV has been recognised as an oncogenic virus, and is implicated in the development of Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's lymphoma and gastric cancer.(66-69)

EBV is also linked to multiple sclerosis (MS), although the mechanism of mediation is unknown. A large Danish study found that the risk of MS increased after IM, regardless of age, gender, severity of infection and time since acute IM.(70) A very recent study examining a military cohort of over ten million people found that although EBV seropositivity is approximately 95% in the general population, EBV seropositivity was almost ubiquitous in patients at the time of MS onset.(71) The risk of MS increased by a factor of 32 following infection with EBV, but this increase was not found following infection with other similar viruses, e.g. CMV. The accompanying editorial article concluded that EBV is likely to be necessary for the development of MS, but that additional factors e.g. genetic susceptibility also play a role.(72)

1.2.10 Vaccine

There have been various attempts to develop an EBV vaccine, with three main potential vaccine options.(41) However, lack of knowledge about EBV incubation period, and immune response during chronic EBV infection has impeded effective vaccine development. It's possible that the recent findings in relation to MS development following IM may help to hasten vaccine progress.

1.3 Clinical condition: GABHS sore throat

GABHS is the most common bacterial cause of acute sore throat, estimated to cause approximately 5-15% of adult cases of sore throat in developed countries, and higher rates in less developed countries.(73) A systematic review from 2000 found rates of between 10-36% of GABHS in adults and children presenting with sore throat.(9)

1.3.1 Clinical Features

A systematic review of nine prospective studies examining the signs and symptoms that were most predictive of sore throat caused by GABHS, concluded that the most predictive variables were: presence of tonsillar or pharyngeal exudate, exposure to GABHS infection in

the previous two weeks, history of fever, and the absence of tender anterior cervical nodes, absence of tonsillar enlargement, or exudate, and absence of cough.(9) No single symptom or sign was deemed to be predictive enough to rule in or rule out GABHS sore throat on its own. A variety of clinical prediction rules (CPRs) exist to aid in the diagnosis and management of GABHS sore throat, the originally CPR being the Centor score.(74) (see also Section 6.1.4)

GABHS sore throat is usually a self-limiting condition; and generally resolves spontaneously (even without antibiotic intervention) by about 7-10 days.(9) However, complications can rarely include sinusitis, otitis media, peritonsillar abscess, rheumatic fever and glomerulonephritis.

1.3.2 GABHS carrier state

Asymptomatic carriage of GABHS is frequent, especially in children. A 2018 systematic review which examined rates of GABHS carriage found a rate of 2.8% of carriage in adults (based on 12 included studies), and 8.0% in children (based on 46 included studies).(75) Because of the overlap of symptoms, patients with acute viral sore throat who have a positive throat swab for GABHS may just be carriers of GABHS and receive antibiotics inappropriately.

1.3.3 Diagnosis and management

A case report from 1999 described two cases of patients presenting to their GP with sore throat and the diagnostic and management approaches that were adopted in each case.(76) The authors posed two questions: whether a throat swab improved the diagnostic accuracy, and whether antibiotics improve symptoms and reduce the risk of complications? This section will address these questions using current evidence.

1.3.3.1 Does a throat swab improve diagnostic accuracy for GABHS?

Most international guidelines do not currently recommend throat swab for diagnosis of GABHS sore throat. However, for research and occasional diagnostic purposes, the reference standard for diagnosis of GABHS is by throat swab culture, despite several issues.(77) This test has a sensitivity of approximately 90%,according to studies that used duplicate throat culture testing.(78) Other advantages are its low cost, acceptability to patients, and the fact that the culture can identify other causative pathogens and guide antibiotic sensitivities.(79) However, throat swab culture results can be controversial as it cannot distinguish carrier state from acute infection.(80, 81) Another disadvantage of the throat swab is that it typically takes more than 24 hours, and often takes several days to obtain the result.

Rapid antigen detection testing (RADT) for GABHS is used in some clinical settings and is currently used more frequently in the USA than in the UK and Ireland. These tests provide a quick indication to the clinician about the presence of GABHS, usually giving a result within 15 minutes. In symptomatic people, they have a sensitivity of approximately 85% and a specificity of 95%, which may make them more appropriate for use as a 'rule in' test.(82, 83) However, they cannot provide information about any other potential bacterial causes of sore throat, which may be identified on the result of a throat swab culture. A recent study piloting pharmacists' use of RADT to test and treat GABHS in Wales resulted in a small reduction in prescriptions for phenoxymethylpenicillin.(84) This was based on 1725 consultations in 56 pharmacies. The pharmacists used a minimum Centor score of 2 or a FeverPAIN score of 1 to offer RADT. Using RADT for diagnosis, 28.2% of participants had positive tests for GABHS and 27.4% of participants were supplied with antibiotics.

The gold standard reference test for GABHS is considered to be serial serum sampling for antistreptococcal antibodies; namely antistreptolysin O titre (ASOT) and antideoxyribonuclease B (ADNaseB). The combination of these two antibodies gives results for GABHS at a sensitivity of 96% and specificity of 89%.(85) However, this is rarely used in practice due to cost, delay and inconvenience for patients and clinicians.

Table 1-3: comparison of available tests for GABHS

Test	Sensitivity (ref)	Specificity (ref)	Turnaround Time
Throat swab culture	90-95% (77)	95-99% (77)	Lab: 2-5 days
RADT for GABHS	85% (82, 83)	95% (82, 83)	POCT: 15 mins
Antistreptococcal antibodies	96% (85)	89% (85)	Serial testing over weeks

1.3.3.2 Do antibiotics improve symptoms and reduce the risk of complications?

A large UK primary care study from 2018 found that sore throat is the condition associated with the highest frequency of inappropriate antibiotic prescribing.(86) A 2021 Cochrane review examining the effects of antibiotics for sore throat (from any aetiology) included 29 trials and 15,337 adults and children with sore throat. The systematic review found that antibiotics provided a modest reduction in the risk of being symptomatic with sore throat (along with headache) and also of developing suppurative and non-suppurative complications.(87) In terms of symptom reduction, the number needed to prevent one sore throat was <6 at day three (but 3.7 for those with GABHS on throat swab) and increased to 18 overall at day seven. They reported that 82% of patients in the control groups are symptom-free by one week (without antibiotic treatment). The authors commented that the number needed to treat for a beneficial outcome may be lower in low-income countries, or in socioeconomically deprived areas of high-income countries, where complications such as acute rheumatic fever are more widespread. There are obvious drawbacks of prescribing antibiotics; antimicrobial resistance, side effects of the medication (including allergy), costs. Another unwanted outcome is that the usually self-limiting presentation of sore throat becomes 'medicalised', resulting in increasing presentation to the GP for future episodes.(88)

1.3.3.3 International guidelines for management of sore throat

Many countries have their own guidelines for the treatment of sore throat, some of which with their most up to date recommendations are summarised below.

The Health Service Executive (HSE) in Ireland issues guidelines in relation to antibiotic prescribing for sore throat.(89) The advice does not mention using throat swab for diagnosis. They advise that most people with sore throat do not benefit from antibiotics and recommend using the FeverPAIN scoring system to aid decisions about antibiotic prescribing.(90) First line recommended antibiotic is phenoxymethylpenicillin 666mg (or 500mg) 4 times daily for 5 days.

The National Institute for Health and Care Excellence (NICE) in the UK and Scottish Intercollegiate Guidelines Network (SIGN) in Scotland have similar guidelines in relation to the management of sore throat.(91, 92) Both recommend against the use of throat swab. Both recommend using the FeverPAIN or Centor score CPR. The NICE guidelines recommend not to offer an antibiotic at Centor score levels of 0,1 or 2, and to consider an antibiotic at levels 3 or 4. The SIGN guidelines recommend not to use antibiotics for sore throat but that 'antibiotics should not be withheld' in severe cases. First line recommended antibiotic is the NICE guideline is phenoxymethylpenicillin 500 mg 4 times a day or 1000 mg twice a day for 5 to 10 days and in the SIGN, guideline is phenoxymethylpenicillin 500 mg 4 times a day for 10 days.

European Society for Clinical Microbiology and Infectious Diseases (ESCMID) guidelines from 2012 suggest using rapid antigen testing if Centor score is 3-4 but advises that the clinical utility of the Centor score is lower in children because of the differing presentations of sore throat in the early years of life.(93)

A set of 2001 guidelines from the USA (recommended by the American Academy of Family Physicians (AAFP), the American College of Physicians–American Society of Internal Medicine (ACP-ASIM) and Center for Disease Control (CDC)), recommend combining the Centor score CPR with rapid antigen testing to guide diagnosis.(80)

1.3.3.4 Duration of antibiotic therapy

A study from 2019 found that Penicillin V four times daily for five days was non-inferior in clinical outcomes to penicillin V three times daily for ten days, in patients with GABHS sore throat.(94) There is no Cochrane review on this topic in adults, although the equivalent review focussing on children under 18 with GABHS sore throat found that shorter courses of oral antibiotics (3-6 days) had comparable efficacy compared to the standard 10-day course of oral penicillin.(95) Current sore throat guidelines from Ireland recommend a 5 day course of antibiotics, and the UK guidelines recommend a 5-10 days course of antibiotics, when they are indicated. Many of the international guidelines have not been updated to reflect this recent research.

A summary of some of the most recent guidelines is presented in Table 1-4.

Table 1-4: comparison of guidelines for the diagnosis and treatment of GABHS sore throat

Guideline	Diagnostic criteria	When to initiate antibiotic treatment	Recommended first line antibiotic
HSE (Ireland) 2021	Clinical and CPR (FeverPAIN score).	Score 2-3: Offer delayed antibiotic. Score 4-5: Offer immediate antibiotic prescription.	Phenoxymethylpenicillin 666mg (or 500mg) four times daily for 5 days.
NICE (United Kingdom) 2019	Clinical and CPR (FeverPAIN or Centor score).	FeverPAIN score 4 or 5, or Centor score 3 or 4: consider an immediate antibiotic or a back-up antibiotic prescription.	Phenoxymethylpenicillin 500 mg four times a day or 1000 mg twice a day for 5 to 10 days.
SIGN (Scotland) 2010	Clinical and CPR (FeverPAIN or Centor score. Throat swabs not to be conducted routinely. They may be used to establish pathogenicity of recurrent severe episodes in adults when considering referral for tonsillectomy.	Antibiotics not to be used routinely. In severe cases, where there is concern about the clinical condition of the patient, antibiotics should not be withheld.	Phenoxymethylpenicillin 500 mg four times a day for 10 days.
ESCMID (European) 2012	Clinical and Centor score CPR	Antibiotics not to be used in less severe cases e.g., 0–2 Centor criteria. In more severe cases, e.g., 3–4 Centor criteria, physicians should consider discussion of the likely benefits with patients.	Penicillin V, twice or three times daily for 10 days.
ACP-ASIM (USA) 2001	Clinical and Centor score CPR. Perform RADT only if Centor score is 2–3	Centor score of 4 or RADT or throat culture positive.	Penicillin V, 500 mg twice or three times daily for 10 days.

RADT = rapid antigen diagnostic test

1.3.3.5 Review of guidelines

A review article from 2007 compared the guidelines from four North American and six European countries and examined the reasons why they differ.(96) The authors found that the guidelines differed with regard to the use of RADT and throat culture and also in terms of recommendation for antibiotics. The review also established that the guideline development method was not clearly stated in most, but that the evidence used to develop the guidelines varied between the North American and European guidelines, with North American guidelines mainly using evidence from authors in the same region. Some of the guidelines did not incorporate landmark studies, or the relevant Cochrane review. Some of the guidelines which recommended antibiotic prescribing to prevent acute rheumatic fever were likely based on old studies from the 1950s, although acute rheumatic fever is now almost unheard of in developed countries. The authors concluded that a clear guideline development method may lead to more consistency in diagnosis and management of sore throat.

An international cross-sectional survey from 2020 examined guidelines for uncomplicated acute sore throat and medical practitioners' perception of best management of this condition in five countries; Australia, Germany, Sweden, UK and USA.(97) The survey (similarly to the review article above) found that there were significant differences between the guidelines of different countries, and that clinicians' perceptions of best management were associated with their country's guidelines. For example, guidelines which recommended throat swabs as part of the management strategy (USA and Sweden) were linked with perceived importance by practitioners working in that country. Because the study was cross-sectional, it was unclear whether guidelines reflected consensus of local practice, or whether local practice was influenced by guidelines. The authors concluded that there should be an effort made in high income countries to reach agreement about the best management of this condition.

Another recent review article examining five guidelines for the diagnosis of GABHS (NICE guidelines, two US guidelines, one German guideline and one from Hong Kong) found that for many of the guidelines, the evidence on which the recommendations were based was not described or was not detailed.(98) The 'Clinical prediction rules' (CPRs) that are incorporated into many guidelines for GABHS diagnosis are discussed in more detail in 6.1.4, and it is clear from the evidence presented in that section, that by using the guidelines with CPRs that are in place currently, it is likely to lead to inappropriate overprescribing of antibiotics.

The surprising differences between guidelines (which should be based on the same underpinning best evidence) has been the case with clinical guidelines for other conditions also. Explanations given for the variations have included insufficient evidence, different interpretation of the evidence, unsystematic guideline development, influence of professional societies, patient preferences, cultural factors and societal factors.(96) The WHO has recommended a systematic process incorporating nineteen key components to ensure that clinical guidelines are based on best available evidence.(99)

1.4 Corticosteroids for sore throat

A BMJ 'rapid recommendation' article from 2017 gave a weak recommendation to use a single dose of oral steroids in cases of acute viral or bacterial sore throat, regardless of severity, for patients aged five and older (excluding those patients in whom IM is suspected or immunocompromised patients, as these were not included in the systematic review on which the recommendation was based).(100) The systematic review of the available evidence included a RCT published in April 2017, which included over 500 primary care patients.(101, 102) The recommendation was stated as 'weak' because of the modest reduction in patients symptoms (intensity and duration of sore throat), and because of variability in patient preferences; shared decision making is recommended.

1.5 Concomitant IM and GABHS sore throat

Previous studies have reported varying rates of GABHS in the presence of IM, and it is difficult to know how much of this is asymptomatic carriage, in the presence of symptomatic IM, as symptoms of both conditions-especially sore throat- overlap. There is scant recent literature in this area; however, a study from 1975 found a rate of GABHS of 30% in 500 cases of IM.(38) The study design and age of participants was not stated. A study of 133 participants from the following year, based in a student health population, found a rate of GABHS of 2.4% in IM cases, and 2.3% in controls.(103) A US study from 1980 reported a rate of 6.2% for positive swab for GABHS in their IM study population of 97 children and adolescents but stated that there was only slight growth (consistent with carrier state) on half of these and so reported the incidence rate for concomitant infection as 3.1%.(104) A further study examining rates of GABHS in 100 cases of IM found a rate of 4%.(105) The rates in these latter studies are similar to rates in studies of asymptomatic GABHS carriage in the general adult population, which can be up to 2.3%, falling with increasing age.(106-108)

1.6 Diagnostic aids

Clinicians often use diagnostic aids within their consultations, either consciously or subconsciously. A number of these are outlined below.

1.6.1 Point of care tests / near patient tests / rapid antigen tests

Point of care tests (POCT) / near patient tests / rapid antigen tests are tests (blood / urine / swab) that are performed during a patient's consultation. They are generally used to detect the presence of a pathogen or an inflammatory marker and they are used as clinical decision aids in some settings.

The only commonly used POCTs in primary care in Ireland up until the Covid-19 pandemic were urine dipstick (in cases of suspected urinary tract infection), and capillary blood glucose testing. RADT are used frequently in north America for the diagnosis of GABHS and have been shown to reduce antibiotic prescribing.(109) However, they are not

recommended as a diagnostic strategy in most European sore throat guidelines. FBC can be used as a POCT also, and the WBC differential count can be useful to distinguish viral from bacterial illnesses, depending on the presenting complaint.

A systematic review from 2019 examining the potential of microbial POCT found that there is considerable possibility for POCT to enhance the management of acute respiratory infections, by providing information about prognosis and assisting with decisions about clinical management (especially in relation to antibiotic prescribing).(110) The authors of this systematic review subsequently published results of a feasibility study (with 93 patients) which assessed the use of a multi-virus rapid POCT in UK primary care.(111) The conclusion of the study was that the POCT was acceptable to physicians and patients, that bacterial infections were over diagnosed at baseline and thus use of the POCT would reduce antibiotic prescribing. However, the POCT did not test for common bacterial respiratory tract infections, and took 65 minutes to obtain results.

Studies examining C-reactive protein (CRP) (an inflammatory marker) POCT have demonstrated reductions in antibiotic prescribing for patients with an infective exacerbation of COPD and for acute uncomplicated lower respiratory tract infection (LRTI).(112-115)

POCT do have some shortfalls nonetheless, as described in a recent editorial article.(116) The testing machines are expensive, and there is additional cost for each individual test that is used. The sensitivity and specificity of POCT for inflammatory markers may not be as accurate in those with milder infections as those with severe infections who are admitted to hospital (as their pre-test probability is lower), and may add little to a primary care consultation.(117, 118) As previously mentioned, if the pre-test probability is low (as would be the case for many POCT in primary care), the more likely it is that a positive test result be falsely positive.(119) Another issue, which may be particularly relevant in terms of self-limiting illnesses such as sore throat, is that in performing POCT, GPs have the potential to medicalise benign clinical presentations.(88) Also, it may be more difficult not to prescribe an antibiotic if a condition has been positively diagnosed by a test, rather than ‘just’ a clinical diagnosis.

1.6.2 'Triage' tests

Another factor to consider when discussing diagnostic testing, is whether an intermediate test ('triage' test), might play a role. A triage test usually is not as accurate as a definitive test but may be used in circumstances where the definitive test is more expensive, takes more time, is more invasive or needs more expertise to interpret.(120) Usually, only patients who obtain a particular result in the triage test will proceed to have a definitive test.

An example of a triage test is the blood test D-dimer, which is used in an emergency department setting and which, if negative, effectively rules out the possibility of the patient having pulmonary embolism and avoids a more invasive, costly definitive test (CT pulmonary angiogram). If the test for D-dimer is positive however, the patient will need to be referred for definitive testing. The triage test needs to have a very high sensitivity, to rule out the diagnosis where missed diagnosis would lead to serious consequences, as in the example of D-dimer and pulmonary embolism. The triage test does not replace the gold standard diagnostic test, rather, it rationalises its use.

The triage test may have a role in conjunction with more definitive tests in the diagnosis of sore throat caused by IM. In the case of IM, an appropriate triage test could be FBC (particularly if available as a POCT). The FBC is a relatively inexpensive, low risk, accessible test, with a quick turnaround time. Depending on the proportion of lymphocytes, and in particular atypical lymphocytes, present in WBC differential, the FBC has excellent specificity for IM, however its sensitivity is much lower (see also Section 1.2.5). This means it may be a suitable test to rule in IM, especially combined with high clinical suspicion, based on symptoms and signs or a CPR. Use of the FBC triage test may avoid the need for more expensive definitive EBV serology testing, which also has a longer lab turnaround time.

1.6.3 Clinical Prediction Rules

'Clinical prediction rules' (CPRs) or 'clinical decision rules' are clinical tools that calculate the independent influence of factors from a patient's history, clinical examination and

diagnostic tests, and stratify patients according to the probability of having the disorder of interest.(121) CPRs are progressively more being used to aid in the diagnostic process and subsequent clinical management decisions.(122) They are often used to help clinicians refine their diagnosis or to 'rule in' or 'rule out' certain conditions depending on the setting in which they are used and the condition they are used for.(123) In primary care they are particularly helpful in ruling out conditions of interest or when adopting an expectant approach to management.

Before they are implemented clinically, CPRs should undergo three stages of development: (i) derivation: factors with predictive power are identified to develop the CPR (ii) validation: the CPR is tested in the original (internal validation) and a new (external validation) population for reliability and accuracy, and (iii) impact analysis: the influence of the CPR may be evaluated in terms of physician behaviour, patient outcomes, or costs.(124) CPRs offer a method of enabling primary care physicians to make clinical decisions which are evidence based. A CPR is deemed to perform well if there is a similar rate between the probabilities calculated from the CPR and the actual outcomes observed, while also differentiating between patients with and without the outcome.(125)

In an article from 2003, the authors describe the limitations of clinical prediction rules, and in particular, barriers to their implementation.(126) They describe that lack of knowledge amongst clinicians or unwieldy, complicated CPRs may be a reason why they are they are not used more widely, that there is no evidence that better predictions equate to superior patient management, and that it can be difficult for clinicians to find the correct model for each scenario. The authors posed the question of whether replacing one CPR with an incrementally better one was like 'building better mouse traps?' They recommended that before a predictive model should be replaced, three questions should be asked; whether the new CPR provides a significant improvement in predicting the outcome of interest, whether using the new CPR will translate into changes in patient management, and whether the new model will be used as often as the older one.

Ideally, a CPR would have 100% sensitivity and specificity, however, generally sensitivity and specificity are mutually exclusive, as one falls the other rises.(122) Preferably, CPRs should

stratify patients into low, moderate and high risk groups for testing and treatment, based on the thresholds for this particular condition; however, thresholds have not been identified for IM.(127) This is discussed in more detail in Section 1.7.3.

1.6.4 Clinical gestalt

The overall clinical impression, or clinical 'gestalt' is an understudied area of clinical medicine. It is described as an intuitive, rather than analytical approach to clinical decision making, based on pattern recognition, which doesn't use a points score or an algorithm to arrive at a diagnosis.(128) It may take into account additional factors that are not usually incorporated into the relevant CPR for that subject area, e.g. high local prevalence rates of a particular illness, a patient's comorbidities, or a recent family history of a contagious pathogen.

Clinical gestalt has been shown to have prognostic value in clinical settings. A 2019 meta-analysis demonstrated that clinician impression performed similarly or better than CPRs, especially for the diagnosis of community acquired pneumonia, acute rhinosinusitis and acute bacterial rhinosinusitis; see Table 1-5.(128) A large 2012 study from Belgium, set in a primary care population, found that acting on gut feeling reduced the risk of missing a serious diagnosis in children.(129) Most of the literature in this area compares gestalt with CPRs, rather than examining the use of gestalt as an element of a CPR.

Table 1-5: diagnostic accuracy of clinical gestalt for specific clinical conditions

(adapted from (128))

Condition	LR+ [e] clinical gestalt (95% CI)	DOR[f] clinical gestalt (95% CI)	LR+ clinical decision rule	DOR clinical decision rule
CAP[a] (adults)	7.7 (4.8-11.5)	14.2 (9.0-21.0)	4.4	7.2
CAP (children)	2.7 (1.1-4.3)	5.5 (1.1-16.0)	N/A	N/A
ARS[b] (adults)	3.0 (2.1-4.4)	8.3 (4.9-13.1)	1.9	3.6
ABRS[c] (adults)	3.9 (2.4-5.9)	13.0 (5.0-27)	2.1	5.9
GABHS[d] sore throat (adults & children)	2.1 (1.6-2.8)	4.6 (2.6-7.8)	1.6 (adults) 2.0 (children)	4.2 (adults) 2.5 (children)

CI = confidence interval
[a] CAP = community acquired pneumonia
[b] ARS = acute rhinosinusitis (diagnosed using any reference standard)
[c] ABRS = acute bacterial rhinosinusitis (diagnosed using antral puncture fluid inspection as reference standard)
[d] GABHS = group A beta-haemolytic streptococcal
[e] LR+ = positive likelihood ratio
[f] DOR = diagnostic odds ratio (positive likelihood ratio / negative likelihood ratio)

1.7 Clinician's approach to diagnosis

1.7.1 Cognitive reasoning

Researchers in the 1970's found that the sequential method of history taking and examination to formulate a differential diagnosis and subsequent final diagnosis (which is commonly taught in medical school) was not what clinicians do in real consultations.(130) It was observed that clinicians generated a working hypothesis (usually in an intuitive manner) early in the consultation which guided a focussed history and examination. This process was called the hypothetico-deductive model, and is now also known as the process of iterative diagnosis.(131)

A 2009 paper on diagnosis in general practice advised that there are three broad stages in reaching a diagnosis: firstly, developing a working hypothesis, secondly, refinement of the hypothesis, and thirdly, reaching a final diagnosis.(132) Using a sample of consultations from

GPs at different stages of their careers, several strategies were identified at each stage. Strategies used in the first stage included spot diagnosis, self-labelling, presenting complaint, pattern recognition trigger. Strategies used in the refinement stage included 'restricted rule outs' (ruling out a short-list of serious diagnoses), stepwise refinement, probabilistic reasoning, pattern recognition fit and clinical prediction rules. Strategies used in the final definition stage included 'known diagnosis' (where the diagnosis is certain to the extent that no further testing is required), ordering further tests, test of treatment, test of time and no diagnostic label applied. In the sample of GP consultations used in this study, the strategies used differed, but the authors could not elucidate whether the variation resulted from the doctors themselves or the case mix.

Probabilistic reasoning, also called Bayesian reasoning (based on Bayes theorem), is used intuitively by clinicians when considering the likelihood of a particular diagnosis in light of a new item of information.(133) It's also used when considering the accuracy of diagnostic tests in their clinical decision making. However, most GPs do not formally recognise their use of probabilistic reasoning.(132) Bayes theorem states that the probability of a hypothesis is revised by additional data, i.e., that the post-test probability equal the likelihood ratio of the test result multiplied by the pre-test probability.(119) Further to that, with a relatively sensitive and specific test, if the pre-test probability is lower, the more likely it is that a positive test result be falsely positive and if the pre-test probability is higher, the more likely it is that a negative test result be falsely negative. Concordant results (low pre-test probability and negative test result, high pre-test probability and positive test result) are likely to be true. Using Bayesian reasoning more formally within a consultation, a CPR could be applied to the patient's pre-test probability to arrive at a post-test probability. This could be further modified by additional information (e.g. laboratory or radiological testing) in order to obtain a more accurate probability of a patient's diagnosis.

1.7.2 Broader context for ordering diagnostic tests

Even when a clinician has performed a thorough history and examination and used a clinical decision aid appropriately to come to an estimate of the patient having the target disorder,

there may be further reasons that influence their decision to perform a diagnostic test. Apart from the obvious diagnostic, therapeutic and prognostic reasons, clinicians order diagnostic tests for a wide range of reasons, which were classified by a 2007 review article into five main categories: diagnostic factors, therapeutic and prognostic factors, patient-related factors, doctor-related factors and policy and organisation-related factors.(134) These factors influence test ordering in different ways, depending on whether the target disorder is being ruled in or ruled out.

If, at the end of the consultation, the clinician feels that the patient has a 'symptom: sign complex' with low post-test probability of a specific condition, it may be the case where no 'diagnostic label' is required or applied, and no diagnostic test is ordered at that time.(135, 136) This happens very often in primary care, and in this instance, the clinician can advise the patient to return for further investigations if symptoms become persistent or worsen. Depending on changes in symptoms and signs at their return visit, the post-test probability may be increased to the test or treat threshold (see also next section).

Specifically, in terms of sore throat consultations, there may be a patient preference for testing-even when it is not clinically indicated- in order to have a definitive diagnosis for prognostic or therapeutic reasons. Because testing for sore throat (blood sample / throat swab / RADT) is relatively low risk, accessible and cheap tests may be undertaken inappropriately in order to maintain a good relationship with a patient or defensively, with fear of litigation in mind.

Several studies have shown that clinical guidelines affect clinicians' test ordering, generally reducing inappropriate tests.(137-139) If a CPR with good sensitivity and specificity could be designed for IM, and incorporated into a clinical guideline, this may reduce inappropriate testing, or allow for more accessible / inexpensive testing options, resulting in improved outcomes for patients along with more prudent use of resources.

1.7.3 Test / treat threshold approach

A test / treat threshold approach may perform an important function in helping clinicians to distinguish between patients in whom they should manage without a diagnostic test or whether they should test the patient.

A 1980 article suggests a 'threshold approach' for clinical decision making.(140) This method is based on the premise that a clinician is faced with three choices when a diagnosis is uncertain; withhold treatment without testing, obtain more data by testing before planning to treat, or treat the patient without testing for the disease. The approach describes assigning values to the 'testing' threshold and the 'test-treatment' threshold. Some CPR studies only divide patients into two risk group; low and high. However, it has been argued that it is more useful to have three risk group categories corresponding to Pauker and Kassirer's threshold model; low ('rule out'), intermediate (obtain more information), and high (manage empirically).(140, 141)

Test and treat thresholds can be affected by a number of factors; properties of the test itself (availability-especially relevant in primary care settings, safety, cost, acceptability to patient), prognosis of disorder, and properties of the treatment (availability, safety, effectiveness).(142) The test threshold is higher with higher risks and cost of test, lower acceptability, less serious target disorder and treatment unavailable, and the converse is also true. The treatment threshold is placed higher when the test itself is safe and cheap, when the condition is less serious if untreated, the treatment is less effective and when the treatment has more adverse effects and vice versa.

A 2015 pilot study similarly described three management options in relation to the patients degree of risk of having a particular disease; 'rule-out', 'test' or 'treat'.(127) Figure 1-1 demonstrates the test and treatment thresholds and suggested management of patients in each risk group. The study found test and treat thresholds of 5% and 55% (in US physicians) for influenza testing and treatment with the anti-viral agent oseltamivir. It also found threshold rates of 4% and 76% (in US physicians) for acute coronary syndrome (ACS). As expected, the test rate for ACS was very low, due to the serious consequences of a missed diagnosis. A study published in 2018 by the same authors examined thresholds in relation to community acquired pneumonia (CAP).(143) In this study, 256 physicians made clinical

decisions about test and treat thresholds for patients with suspected CAP. The results showed that the physicians consistently overestimated the likelihood of CAP, with the test and treat thresholds for the group overall being determined at 9.5% and 43.1% respectively.

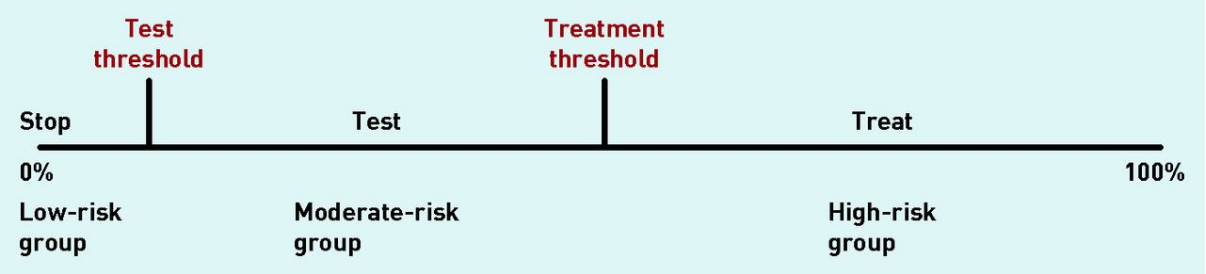

Figure 1-1: the threshold model of decision-making (reproduced with permission from (127)).

The test and treat thresholds have not been determined for most conditions, including IM and GABHS. Hypothetically, as the nature of the test for both these conditions is relatively cheap, readily available and low risk, the test threshold for both conditions should be low, possibly similar to that found in the study for influenza (5%).(127) Likewise, one would imagine that the treatment thresholds for these two conditions (above which the clinician is relatively certain of the diagnosis, and treats the condition without further testing) should be similar to other acute respiratory illnesses at approximately 50% (55% for influenza, 43% for CAP), although IM can rarely have life threatening complications, which may raise the treatment threshold, and produce a large moderate risk group in whom testing is advised.

1.8 Thesis study aims

This thesis aims to further research in the area of improving the diagnosis and management of sore throat in young adults, especially sore throat caused by IM. There were four broad objectives;

I. To systematically review the effectiveness of antiviral therapy for infectious mononucleosis (IM) (this was originally completed and published in the Cochrane Database of Systematic Reviews; with searches updated for this thesis).(144)

II. To derive and externally validate a clinical prediction rule (CPR) to aid in the positive diagnosis of IM caused by EBV.

III. To validate the Centor score CPR in an Irish setting.

IV. To explore potential associations between IM caused by EBV and fatigue and time missed from college / work in younger adults.

1.9 Thesis outline

This thesis is presented in eight chapters. Chapter 2 presents the process of systemically reviewing the evidence examining whether antiviral agents are effective in the treatment of infectious mononucleosis. Chapter 3 presents the methodology of the establishment and prospective follow-up of the cohort utilised as the basis of this thesis research. Chapter 4 presents the process of deriving and validating a clinical prediction rule to be used in the diagnosis of IM caused by EBV, and the results of externally validating the EBV CPR in a US population are presented in Chapter 5. In Chapter 6 the results of validating the Centor Score CPR in an Irish study population are presented, and Chapter 7 presents the results of a six month follow up of the study cohort. Finally, Chapter 8 summarises the findings and discusses clinical and research implications of this thesis.

2 Chapter 2: systematic review: to examine the effectiveness of antiviral agents for infectious mononucleosis

This thesis chapter is an update of a Cochrane review which was first published in 2016.(144) The search for studies was updated on 8th December 2021, although no new studies were identified.

2.1 Background

2.1.1 Description of the condition

Infectious mononucleosis (IM) is a clinical syndrome that is characterised by lymphadenopathy, fever and sore throat.(10) In developed countries, approximately ninety per cent of cases are caused by the Epstein-Barr virus (EBV), about 5% to 7% are caused by cytomegalovirus (CMV) and less than 1% are caused by *Toxoplasma gondii*.(46) This proportion varies in areas of deprivation and in the elderly, and 'mononucleosis-like illness' can also be caused by non-infectious causes.(10) EBV is a widespread virus that is transmitted primarily through infected saliva.

EBV infection may be subclinical during childhood years, without causing the overt syndrome of IM. However, the incidence of symptomatic infection rises in adolescents and adults, and studies have shown that EBV eventually infects over 95% of adults.(44) The overall incidence of IM in the United States is about 500 cases per 100,000 people per year.(44) In high-income countries, incidence of IM peaks in the late teens and falls after the age of 35.(13) By contrast, in low-income countries most children are infected with EBV before they reach adolescence and symptomatic IM is uncommon.(14)

Following infection with EBV, the incubation period is four to eight weeks.(145) Symptoms of IM usually peak one week from onset, and generally start to resolve over the next one to four weeks.(26, 27) Occasionally, fatigue after acute IM can be severe and persistent. Persistent fatigue was present in 12% of cases at six months after illness onset in one prospective study and another cohort study found that 9-22% of patients with IM were

classified as having Chronic Fatigue Syndrome six months after the acute illness.(61, 62) The virus can continue to shed in saliva for a median duration of six months.(24)

IM is regarded as a benign disease in the majority of cases and is associated with typical features of fever, sore throat, lymphadenopathy, fatigue and atypical lymphocytosis.(44)

IM Diagnosis

IM is usually diagnosed clinically, based on characteristic signs and symptoms. Typical features of IM include lymphadenopathy, fever, sore throat and fatigue. However, there are no definitive diagnostic criteria. Blood testing during acute IM usually reveals atypical lymphocytosis and abnormal liver function tests (LFTs).(37)

Laboratory tests are usually not required for diagnosis. However, specific antibody tests may be required to confirm diagnosis or to identify the cause of illness in atypical cases. A definitive diagnosis can be made by testing for IgG and IgM antibodies against viral capsid antigens, early antigens and EBV nuclear antigen proteins.(44) Recent studies have proposed a number of biomarkers for monitoring disease severity in IM caused by EBV.(146, 147)

IM Complications

IM can be associated with a variety of complications affecting multiple organ systems. As previously mentioned, fatigue after IM can be prolonged. Haematological complications are observed in 25% to 50% of cases and are generally mild.(44) Rare complications, such as airway occlusion secondary to oedema of the soft palate and tonsils and peritonsillar abscess, can occur.(148) Upper airway obstruction is seen in approximately 1% of cases.(44) Splenomegaly is seen in approximately 50% of patients with IM but usually begins to resolve by the third week of the illness.(31) Splenic rupture is rare - occurring in 0.1% to 0.2% of cases - but potentially fatal.(149, 150) EBV has also been associated with other complications including pneumonia, pleural effusions, hepatitis and cholestasis, myocarditis and cardiac conduction abnormalities, acute renal failure, and (in 1% to 5% of cases) neurological complications.(44, 151-153)

IM Treatment

IM is generally self-limiting, and there is no specific treatment. The mainstay of treatment for IM is supportive care. Patients should be encouraged to maintain adequate fluid and nutrition intake. Over the counter medications are recommended to relieve symptoms of sore throat, fever and malaise.(44) Corticosteroids may be used in the treatment of complications. However, a Cochrane review evaluating the effectiveness of corticosteroids for the control of symptoms concluded that there was insufficient evidence of clinical benefit.(53) Metronidazole, an anaerobic antibacterial agent, has recently been studied in severe cases of patients with IM who were hospitalised and found to reduce hospital stay.(55) Antiviral medications have been used to treat IM, but the use of antiviral agents for IM is controversial.

2.1.2 Description of the intervention

Antivirals for IM have been studied previously in a meta-analysis of five randomised controlled trials of acyclovir for the treatment of IM.(52) This 1999 systematic review showed less oropharyngeal EBV shedding at the end of therapy but failed to show a clinical benefit in terms of sore throat, weight loss and absence from school or work compared to placebo. A randomised pilot study comparing valacyclovir with no treatment in young adults with IM showed a transient reduction of oropharyngeal EBV shedding during therapy and a decrease in the number and severity of reported symptoms in the valacyclovir group, but with no difference between the two groups in the peripheral blood EBV load.(154)

2.1.3 How the intervention might work

There are several antiviral agents, but the two that have been studied most with respect to IM are acyclovir and valacyclovir. Acyclovir is a nucleoside analogue that selectively inhibits the replication of certain viruses. It is active against herpes simplex virus types 1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV) and Epstein-Barr virus (EBV). Valacyclovir acts as an

oral prodrug and is converted in vivo to acyclovir. Other antiviral agents that have been shown to have in vitro activity against herpes viruses are penciclovir, famciclovir, ganciclovir, valganciclovir, cidofovir and foscarnet.(155) All these agents act by preventing viral replication by inhibiting viral DNA synthesis. This helps to keep the virus inactive.

Antiviral medications are generally well tolerated. However, the most commonly reported side effects of acyclovir (observed in between 1/10 and 1/100 of cases) are nausea, vomiting, diarrhoea and abdominal pain, headache, dizziness, fatigue and fever, as well as skin rashes (including photosensitivity and itching).(156)

2.1.4 Why it is important to do this review

A general practice with 10,000 patients can expect to see approximately seven new cases of IM per year.(157) Although generally not considered a serious illness, IM can lead to significant loss of time from school or work due to profound fatigue, or the development of chronic illness.(26, 157) Also, in rare cases, it can lead to potentially life-threatening complications such as splenic rupture, encephalitis and severe upper airway obstruction.(54) If the incidence of complications could be reduced, by implementing evidence-based treatment, it would impact positively on patient care. These complications also have economic implications - both in terms of healthcare costs and loss of productivity. As such, there is great interest in developing regimens for treating IM with antiviral agents.

Antiviral medications are known to be expensive. Another consideration is the emergence of resistance to antiviral agents. There needs to be an evidence base for using these medications so that the available resources are used efficiently and effectively. There are no professional society guidelines for the management of IM. This indicates a lack of clarity regarding the current evidence in relation to antiviral treatment for IM. It was hoped that the findings of this review would inform the preparation of a clinical guideline or policy document.

The previous meta-analysis examining acyclovir for the treatment of IM showed some benefit in reducing oropharyngeal EBV shedding but no evidence to support its clinical effectiveness.(52) These data are now over 20 years old and only included one antiviral agent, acyclovir. It was felt that was necessary to search, appraise and summarise randomised controlled trials (RCTs) of antiviral agents for IM.

2.2 Objectives

To assess the effectiveness of antiviral therapy for the treatment of infectious mononucleosis (IM).

2.3 Methods

2.3.1 Criteria for considering studies for this review

2.3.1.1 Types of studies

In order to reduce the risk of bias, only randomised controlled trials (RCTs) that examine the benefits and side effects of antiviral medication in infectious mononucleosis were included. Non RCT study designs were not included.

2.3.1.2 Types of participants

Immunocompetent participants of any age or sex with both clinical and confirmed laboratory diagnosis of infectious mononucleosis, who have had symptoms for 14 or fewer days. Laboratory diagnosis is by Monospot test or atypical lymphocytosis or EBV-specific serology.

2.3.1.3 Types of interventions

Antiviral medication (acyclovir, valacyclovir, penciclovir, famciclovir, ganciclovir, valganciclovir, cidofovir and foscarnet) used for any duration or at any dose or by any route of administration. RCTs comparing antivirals with placebo, or no treatment were included. RCTs were excluded if they only compared two different antivirals, with no placebo / no treatment arm.

2.3.1.4 Types of outcome measures

Primary outcomes

1. Time to clinical recovery.
2. Adverse events and side effects of medication: as reported in the original studies by patients and clinicians.

Secondary outcomes

1. Time to resolution of abnormal clinical examination (as assessed clinically by physician). This included fever (> 37.5 degrees C), sore throat, lymphadenopathy, splenomegaly and hepatomegaly.
2. Development of complications of infectious mononucleosis.
3. Viral shedding (as reported in the original studies).
4. Psychosocial outcomes:
    a. Health-related quality of life (as reported in the original studies).
    b. Days missing from school or work.
5. Economic outcomes: based on collecting cost data from studies, where available.

2.3.2 Search methods for identification of studies

2.3.2.1 Electronic searches

The Cochrane Central Register of Controlled Trials (CENTRAL, latest issue) was searched. This contains the Cochrane Acute Respiratory Infections (ARI) Group's Specialised Register. MEDLINE (1946 to December 2021), EMBASE (1974 to December 2021), CINAHL (1981 to December 2021), LILACS (1982 to December 2021) and Web of Science (1955 to December 2021) were also searched. The search strategy below was used to search MEDLINE and CENTRAL. The MEDLINE search was combined with the Cochrane Highly Sensitive Search Strategy for identifying randomised trials.(158) The search strategy was adapted to search the other databases. There were no restrictions imposed in terms of language, publication date or publication status on the electronic database searches.

MEDLINE search strategy

1. Infectious Mononucleosis/
2. mononucleosis.tw.

3. glandular fever.tw.
4. Epstein-Barr Virus Infections/
5. Herpesvirus 4, Human/
6. ((epstein-Barr or epstein Barr) adj2 (virus* or viral*)).tw.
7. ebv.tw.
8. or/1-7
9. exp Antiviral Agents/
10. antiviral*.tw.
11. antivirus*.tw.
12. exp Acyclovir/
13. (acyclovir or aciclovir).tw,nm.
14. (valacyclovir or valaciclovir).tw,nm.
15. (gancyclovir or ganciclovir).tw,nm.
16. (valganciclovir).tw,nm.
17. (cidofovir).tw,nm.
18. (foscarnet).tw,nm.
19. (penciclovir).tw,nm.
20. (famciclovir).tw,nm.
21. or/9-20
22. 8 and 21

2.3.2.2 Searching other resources

The World Health Organization International Clinical Trials Registry Platform (WHO ICTRP) (www.who.int/ictrp) and ClinicalTrials.gov were searched for completed and ongoing trials. The reference lists of included trials were searched to ensure that the main search had not missed any trials. The authors of included trials (that were published in the last 15 years) were contacted for the purpose of identifying missing trials. The European Medicines Agency (EMA) was contacted to request clinical study reports for any relevant trials.

2.3.3 Data collection and analysis

2.3.3.1 Selection of studies

All titles and abstracts retrieved to assess eligibility against the inclusion criteria, as well as to identify multiple reports from single studies. Full-text copies of all papers considered to be potentially eligible were obtained. These were independently assessed by Muireann de Paor and Susan Smith in terms of suitability for inclusion. Disagreement was resolved by discussion and, where necessary, asking the opinion of a third reviewer (Tom Fahey). The authors of a number of the primary studies were contacted for clarification. Any papers that did not meet the inclusion criteria were excluded.

2.3.3.2 Data extraction and management

Data extraction was completed using a standard data extraction form.

2.3.3.3 Assessment of risk of bias in included studies

Risk of bias was assessed using the tool described in the *Cochrane Handbook for Systematic Reviews of Interventions.*(159) The risk of bias according to the following domains.

1. Random sequence generation (selection bias)
2. Allocation concealment (selection bias)
3. Blinding of participants and personnel (performance bias)
4. Blinding of outcome assessment (detection bias)
5. Incomplete outcome data (attrition bias)
6. Selective reporting (reporting bias)
7. Other bias (other sources of bias related to particular trial design (cross-over and cluster-randomised) or specific circumstances)

The risk of bias was classified as: low risk, high risk or unclear risk of bias.(159) An overall risk of bias assessment graph displaying the review authors' judgements about each risk of bias item was presented as percentages across all included studies.

Where necessary, study authors were contacted for clarification. Any disagreement was resolved by discussion between the two review authors (Muireann de Paor and Susan Smith) and, where necessary, a third review author (Tom Fahey).

2.3.3.4 Measures of treatment effect

Treatment effect was measured by using either dichotomous data or an ordinal rating scale. For continuous data, the MD or standardised MD was calculated (using the method described in this paper) where different measures were used.(160)

2.3.3.5 Unit of analysis issues

The individual was considered the unit of analysis. If any non-standard design RCTs were identified, it was planned to follow the guidance in the *Cochrane Handbook for Systematic Reviews of Interventions.*(159)

2.3.3.6 Dealing with missing data

Lead study investigators or corresponding authors were contacted for missing trial data, along with data missing from published reports and for additional clarification. Three authors responded with additional information but only one was in a position to provide selected original data.

For data reported as median and range, the method described in Hozo et al was used to convert it to mean and standard deviation,(160) however, for skewed data (eg time to event), a non-parametric statistical method may have advantages over parametric methods, in terms of validity Where there was data missing from a study, this was explicitly stated and reported in the 'Risk of Bias' table. The potential impact of missing data on the review findings was discussed in the Discussion section.

2.3.3.7 Assessment of heterogeneity

Included studies were assessed for clinical heterogeneity. Minimal data was pooled for analysis across trials as there was diversity in intervention (which drug was administered, route of administration, co-administered medication, use of placebo), outcomes (which outcomes were reported at which time points and whether they were continuous or dichotomous outcomes) and length of follow up.

2.3.3.8 Assessment of reporting biases

Reporting bias was minimised by conducting a comprehensive search for studies that met the eligibility criteria, including grey literature and unpublished trials, and by contacting trial authors for missing information. It was planned to assess the potential for publication bias in funnel plot analysis if there was sufficient and appropriate trial data to combine.

2.3.3.9 Data synthesis

The trial outcomes were too heterogeneous to perform a meta-analysis to calculate a weighted intervention effect for the primary outcome across trials. The results of some of the studies were pooled where appropriate. The statistical analyses were performed using Review Manager software.(161)

A 'Summary of findings' table (Table 2-2) was prepared to present the results for each of the outcomes, including adverse effects, as outlined in Section 2.3.1.4 'Types of outcome measures' (with results synthesised mainly narratively). The five GRADE considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) were used to assess the quality of the body of evidence as it relates to the studies that contribute data to the meta-analyses for the prespecified outcomes. The GRADE approach specifies four levels of quality. The highest quality rating is for randomised trial evidence. However, randomised trial evidence may be downgraded to moderate, low or even very low quality evidence, depending on the presence of the above five considerations.(159)

The GRADEpro software was used to prepare the table.(162)

2.3.3.10 Subgroup analysis and investigation of heterogeneity

It was planned to pursue subgroup analyses based on patient age, setting and placebo versus no treatment controls with sufficient data but unfortunately this was not possible.

2.3.3.11 Sensitivity analysis

It was planned to carry out sensitivity analysis to explore the impact of risk of bias on study findings, but all included studies were at a moderate or high risk of bias, so this was not possible.

2.4 Results

2.4.1 Description of studies

See Section 2.5.1.

2.4.2 Results of the search

See Figure 2-1 for study flow diagram.

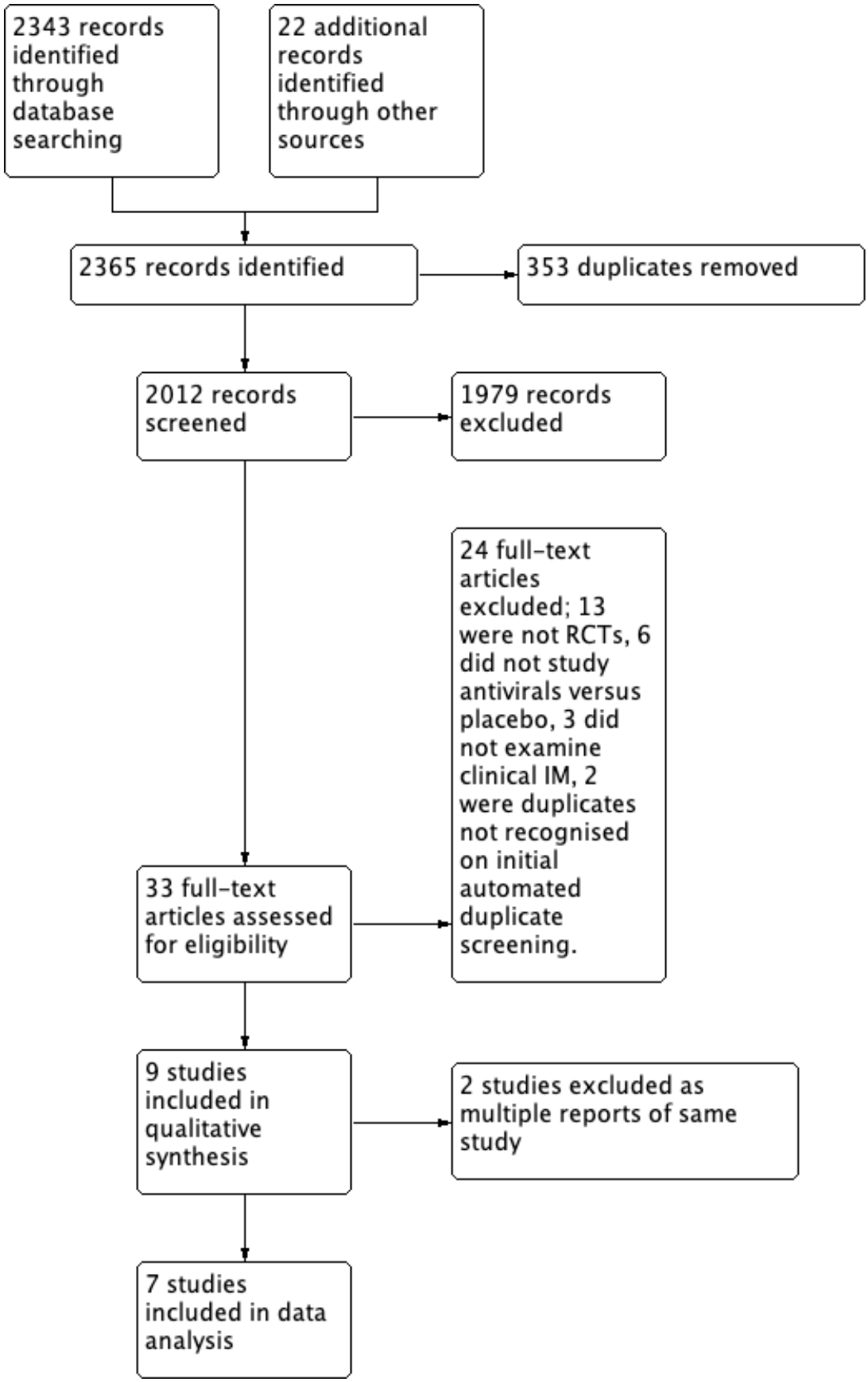

Figure 2-1: PRISMA flow diagram of included studies

A total of 2343 abstracts were obtained from electronic searches. An additional 22 studies were found from searching other sources. From the screening of titles and abstracts, 33 studies were found to be potentially relevant. On full text retrieval 24 studies were excluded, leaving nine to be analysed. Three of these appeared to be different reports of the same study.(163-165) An attempt was made to contact the authors for confirmation of this, but no response was received. Andersson 1986 was used as the main paper for this trial, thus leaving seven included studies.(164)

The seven studies found were those included in the 2016 Cochrane review.(144) However, two ongoing trials were found in the updated December 2021 search.(166, 167) Neither of these trials have published protocols, but some information about them can be accessed from trials registry platforms (ICTRP and Iranian Registry of Clinical Trials (IRCT)). Both trials were registered in February 2021. The first trial is based in China and includes children aged one to eighteen, who meet particular diagnostic criteria of IM (as formulated by the infectious diseases group of Chinese Medical Association and the National Children's Epstein Barr virus infection cooperation group in 2016). It has three treatment arms with 212 participants in each group; acyclovir, ganciclovir and control. It is unclear whether the trial is based in a hospital or primary care setting. The primary outcome is stated as duration of fever. The second trial is a publicly funded randomised double-blind trial based in Iran. It includes 46 hospitalised children aged one to sixteen with symptoms of IM (including sore throat, lymphadenopathy and fatigue). It has two treatment arms; acyclovir and placebo. Primary outcomes include reduced severity of disease (fever, sore throat, respiratory distress, stridor) and reduced duration of hospitalisation. Recruitment for this trial has now concluded, however, the trial results have not yet been published.

2.4.3 Included studies

Seven trials were included and are summarised in Section 2.5.1.(154, 164, 168-172)

2.5 Characteristics of studies

2.5.1 Characteristics of included studies

Table 2-1: Characteristics of included studies gives further information about the included studies.

Table 2-1: Characteristics of included studies

Study	Methods	Participants	Interventions	Outcomes	Notes
Andersson 1986(164)	Double-blinded RCT	33 hospitalised participants (aged 15-25) with symptoms of infectious mononucleosis for one week or less. Two participants excluded because of a change in diagnosis to tonsillitis due to primary herpes simplex or group A strep.	Acyclovir (ACV) 10mg/kg IV 8hrly for 7days versus placebo.	Assessment of general health by patient and physician, duration of recovery as assessed by patient and physician, duration of weight loss, duration of fever > 37.5, duration of sore throat, duration of tonsillar swelling, duration of rash, duration of lymphadenopathy, duration of enlargement of liver and spleen, time away from school / work. Time to normalize WBC count, atypical lymphocytes, liver enzymes. Serological and virological measurements taken also.	Three reports seem to be from the same trial: Andersson 1985, Andersson 1986 and Ernberg 1986. 11/15 of the ACV group and 9/16 of the placebo group had antibiotics pre admission.
Andersson 1987(168)	Double-blinded RCT	60 adults (aged 15-30) hospitalised with symptoms of infectious mononucleosis for one week or less. Two participants excluded because of a change in diagnosis and two excluded for not complying with medication.	Acyclovir 800mg 5 times daily po for 7days versus placebo.	Duration of: recovery as assessed by patient and physician, weight loss, fever > 37.5, difficulty swallowing, sore throat, tonsillar swelling, tiredness, abdominal pain, liver enlargement, elevated liver enzymes, spleen enlargement, absence from school / work, atypical monocytes > 5%, absolute lymphocytosis > 50%. Number of patients with increased serum creatinine during treatment, number of patients with positive monosticon test after six months. Virological outcomes also measured.	3 patients in Acyclovir group required IV Acyclovir and prednisolone for 10 days, whereas none of the placebo group had this treatment Virological findings were only provided for 36 out of 56 patients.

Study	Methods	Participants	Interventions	Outcomes	Notes
Balfour 2007 (169)	Double-blinded RCT	23 students (aged over 15) with symptoms of infectious mononucleosis for two weeks or less. Setting unclear.	Valomaciclovir 2 grams orally twice daily for 21 days, versus placebo.	Number of participants with improvement in clinical symptoms and reductions in viral burden from baseline, number of participants who experienced adverse events.	Unpublished study: data obtained from conference slides supplied by author and information on Clinicaltrials.gov
Balfour et al 2007(154)	Pilot study	20 students (aged over 18) with symptoms of infectious mononucleosis for one week or less. Setting: student health centre.	Valacyclovir 1g po every 8 hours for 14 days or no antiviral drug (no placebo given).	The primary outcome was the proportion of subjects with laboratory confirmed primary EBV infection who had ≥2 log10 decrease in EBV copies/mL in oral washes during the treatment period. Secondary outcomes included clinical effects.	This is the same study as 'Controlled Trial of Valacyclovir in Infectious Mononucleosis' found on Clinicaltrials.gov
Pagano 1983(170)	Double-blinded RCT	20 adults with relatively severe symptoms of infectious mononucleosis requiring hospitalisation.	Acyclovir (ACV) 500mg/m2 IV 8hrly for 5 days versus placebo.	Unclear whether clinical findings were reported by patient or physician. No table of results for clinical findings. Clinical findings: sore throat, splenomegaly, lethargy, lymphadenopathy, temp, return to baseline body weight. Oropharyngeal EBV shedding measured from 4 participants in each group. Spontaneous outgrowth of EBV-infected B lymphocytes. Complications and drug reactions not mentioned.	Unclear whether patients had symptoms for 14 days or less. Two patients in the placebo group and one in the ACV group had steroids.
Simon 2003(171)	Double-blinded three	45 children (aged 2-18) with symptoms of	1. valacyclovir 20mg/kg tds	Sore throat, stomach ache, fatigue, swollen glands, headache, vomiting, rash, loss of	

Study	Methods	Participants	Interventions	Outcomes	Notes
	arm pilot RCT	infectious mononucleosis for one week or less. Clinic setting.	for 14 days and placebo A od for 5 days. 2. valacyclovir 20mg/kg tds for 14 days plus prednisolone 1mg/kg/day for 5 days. 3. placebo B tds for 14 days plus placebo A od for 5 days.	appetite, nausea, sweats, chills, swollen eyes, runny nose, cough, and feeling bad were scored as either absent (0), mild (1), moderate (2), or severe (3).	
van der Horst 1991(172)	Double-blinded RCT	132 participants with a clinical and laboratory diagnosis of infectious mononucleosis. Setting unclear. Symptoms present for one week or less.	Acyclovir 600mg po 5 times daily for 10 days versus placebo.	Clinical outcomes were measured on days 3,5,10,30. Temperature, weight loss, sore throat, lymphadenopathy, hepatic and splenic enlargement were weighted and scored according to severity. Hours of bed rest, time to return to normal activities, appetite were also recorded. Virological outcomes were measured on days 0,10, 30.	'inadequate handling' of samples reported in study. Also 12 patients dropped out.

2.5.1.1 Intervention

Six of the seven studies explored the effects of antivirals versus placebo and one trial compared antiviral treatment with no drug.(154) The antivirals examined were Acyclovir, Valomaciclovir and Valacyclovir. One of the trials had a third study arm which compared antiviral and steroid to placebo.(171)

The dose of antiviral, route of administration, and duration of treatment varied between the trials.

In the trial Andersson 1987, three patients in the treatment group required IV antiviral and steroid for ten days, whereas none of the patients in the placebo group had this treatment.(168) In the study Andersson 1986, 11 of 15 of the treatment group and 9 of 16 of the placebo group had antibiotics pre admission.(164) In the study Balfour et al 2007, some participants had oral steroids co-administered, but it is not reported how many from each group had this.(154) In the study Pagano 1983, two patients in the placebo group and one in the treatment group had steroids co-administered.(170)

2.5.1.2 Setting

Two of the seven studies were conducted in Europe while the other five took place in the USA.(164, 168) The European trials along with the Pagano 1983 study took place in an inpatient setting. The studies Balfour et al 2007 and Simon 2003 took place in outpatient settings with the former set in a student health centre and the latter in a paediatric clinic. The trial setting was unclear in two of the studies.(169, 172) All trials were undertaken by researchers either located in hospitals or at academic institutions.

2.5.1.3 Participants

Participants ranged in age from young children (aged from 2) to young adults (although no maximum age was specified in the exclusion criteria of most of the trials). In the six trials that reported gender there were consistently more males than females. One trial did not report the participants' ages.(172) One trial did not report the gender of participants.(170)

One of the inclusion criteria for this review were that diagnosis of IM was based on clinical symptoms and laboratory parameters. The diagnostic laboratory tests used in the studies included positive heterophile test, Monospot test, atypical lymphocytosis, and EBV antibody testing. Four studies used both heterophile test and EBV antibody testing.(164, 168-170) Although not explicitly stated, the study report suggests that the heterophile test was done first, and if this was positive participants had EBV antibody testing prior to inclusion. In the study Van der Horst 1991 the participants had to have one of: atypical lymphocytosis, positive heterophile test or positive EBV IGM antibodies for initial consideration for trial inclusion, and all had EBV antibody testing prior to inclusion. In two studies EBV antibody testing alone was used.(154, 171) The inclusion criteria also specified that symptoms should be present for 14 days or less and that participants should be immunocompetent. In the trial Pagano 1983 the duration of symptoms and immunocompetence of the participants was unclear. The average time from symptom onset to trial inclusion was variably reported; Andersson 1987 reported the time from clinical onset to treatment, but it was unclear whether this was trial treatment or otherwise, Andersson 1986 reported number of days with symptoms before admission but again it was unclear as to whether admission referred to trial inclusion or hospitalisation. The studies Balfour 2007 and Balfour et al 2007 reported number of days ill at baseline.

2.5.1.4 Outcomes and follow-up assessment

Outcomes examined to evaluate the effectiveness of antivirals were quite heterogenous between studies.

One of the primary outcomes-Time to clinical recovery- was reported in three of the seven included studies. In the studies Andersson 1986 and Andersson 1987 it was reported as the number of days; whereas the study Van der Horst 1991 reported the outcome in a dichotomous way; as recovery by day five or ten.

Adverse events and side effects of medication (which was the other primary outcome) was reported narratively by five of the studies.(154, 164, 168, 169, 172)

Viral shedding was the most evaluated outcome, reported by six of the trials. Other outcomes reported by more than one study included: Time to resolution of abnormal

clinical examination (164, 168, 171), Development of complications (164, 168, 169), Days missing from school or work (164, 168).

Outcomes were assessed at different times in the different studies. The length of follow up was not clear from some of the studies and it was inferred from information in results tables etc. Follow up varied from 20 days (171), 35 days (172), 120 days (170), 170 days (154) to six months (164, 168, 169).

2.5.2 Excluded studies

Studies were excluded for a variety of reasons based on study design and intervention criteria. 22 full-text articles were excluded; 12 were not RCTs, 5 did not study antivirals versus placebo, 3 did not examine clinical IM, and 2 were duplicates not recognised on initial automated duplicate screening.

2.5.3 Risk of bias in included studies

All seven included studies were at either unclear or high risk of bias. The 'Risk of bias' summary and 'Risk of bias' graph are presented in Figure 2-2 and Figure 2-3 respectively.

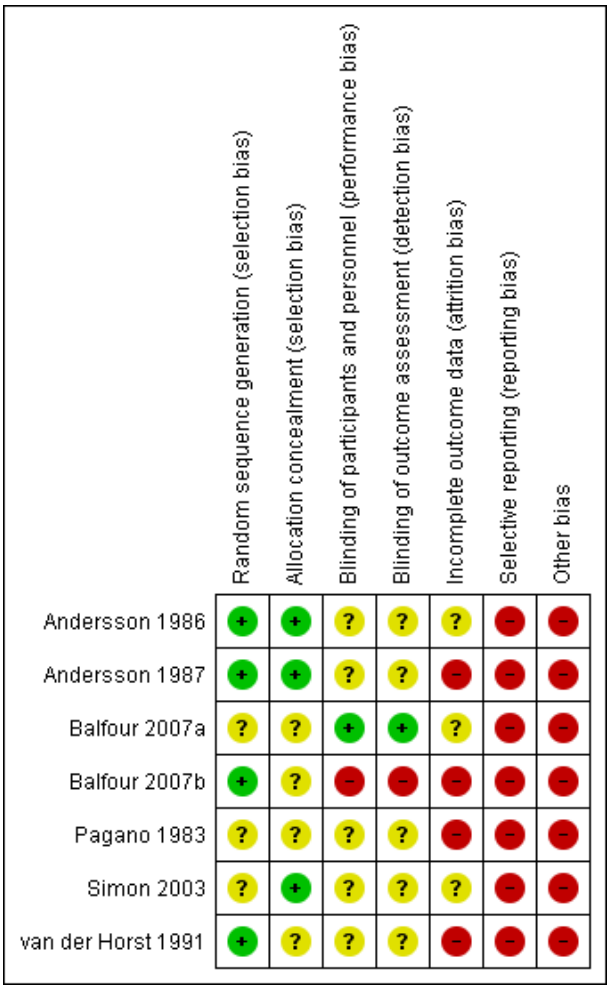

Figure 2-2 Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

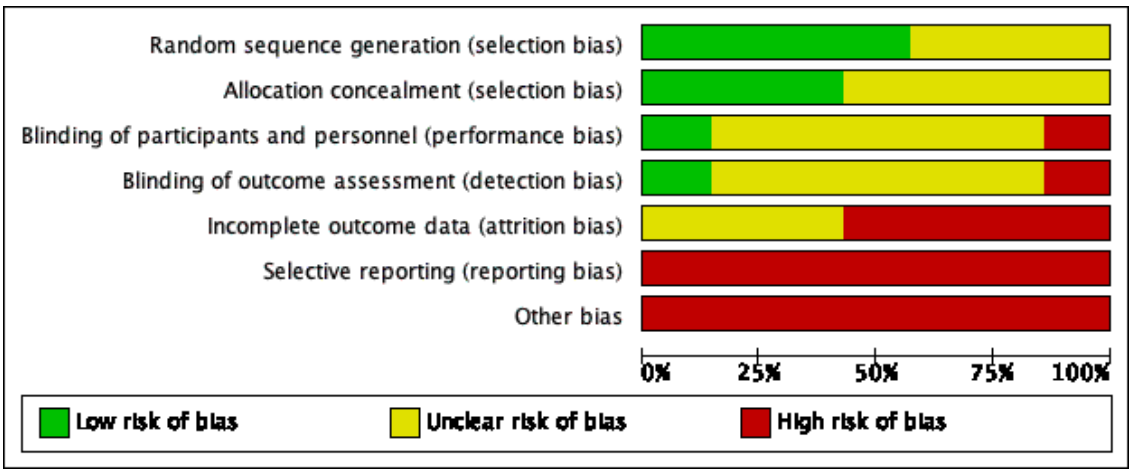

Figure 2-3 Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

2.5.3.1 Allocation (selection bias)

Three trials (164, 168, 171) provided adequate details on allocation concealment, in the remaining four (154, 169, 170, 172) this was unclear.

2.5.3.2 Blinding (performance bias and detection bias)

Five of the trials were 'double-blinded' but did not specify who was blinded.(164, 168, 170-172) In the trial Balfour 2007 details were found on clinicaltrials.gov specifying that subject, caregiver, investigator and outcomes assessor were blinded. In Balfour et al 2007 clinical observers and participants were not blinded.

2.5.3.3 Incomplete outcome data (attrition bias)

In three trials (168, 170, 172), there was a high risk of attrition bias and in the remaining four trials (154, 164, 169, 171) there was an unclear risk of attrition bias.

2.5.3.4 Selective reporting (reporting bias)

The risk of selective reporting in all included trials was high.

2.5.3.5 Other potential sources of bias

In the trial Andersson 1986 some patients received IV fluids and some also had antibiotics before the trial. In the trial Andersson 1987 there were differences in severity of illness between patients and three patients who had IV rather than oral antiviral and steroids were included in the analysis. In Balfour et al 2007 some participants had oral steroids co-administered, but it is not reported how many from each group had this. Balfour 2007 is an unpublished trial, and therefore not peer reviewed. In Pagano 1983 it was unclear whether patients had symptoms for 14 days or less, and also, two patients in the placebo group and one in the treatment group had steroids. In Simon 2003, the study funding source and conflicts of interest were not stated; however, two of the authors were from a pharmaceutical company. The groups in the trial Van der Horst 1991 had significant baseline imbalances in that more acyclovir recipients had a temperature >37.5°C, and the mean sore throat score was slightly greater for acyclovir recipients.

2.5.4 Effects of interventions

Seven RCTs were included in the review. The trials dated from 1983 to 2007 and were heterogeneous in terms of outcome assessment and how they were reported, therefore few of the results of the trials were pooled. There were statistically significant improvements in the treatment group for two of the twelve outcomes.

See 'Summary of Findings' in Table 2-2 and 'Data Analysis' in Table 2-3 below.
The main results are summarised here, with further details outlined in the subsequent sections.

The primary outcome 'Time to clinical recovery' was reported by three studies. Two of the studies examined this as assessed by physician and as assessed by patient.(164, 168) When the results of recovery 'as assessed by physician' were pooled, there was a mean reduction in number of days of 4.56 days in the treatment group with wide confidence intervals [-8.04, -1.08]. When the results of the same two studies were pooled for the outcome 'as assessed by patient' this did not yield any statistically significant result. The study Van der Horst 1991 also reported this as a dichotomous outcome, with recovery by day five and by day ten. There was no statistically significant difference found at either time point. This outcome was not reported by the other studies.

Trial results for the other primary outcome 'adverse events and side effects of medication' could not be pooled due to the potential for double counting results. These results were reported in five of the seven studies and the majority of trials reporting this outcome did not find any significant difference between treatment and control groups.

In terms of secondary outcomes, results from two studies were pooled for the outcome 'Time to resolution of lymphadenopathy', with a mean reduction in number of days of 8.94 [-11.75, -6.14] in favour of the treatment group.(164, 171)

The overall effect on viral shedding from the six studies that reported this outcome was that viral shedding was suppressed while on antiviral treatment, but this effect was not sustained when treatment was stopped.

For the eight other outcomes reported in included studies there was no statistically significant effect of anti-viral therapy in IM.

Table 2-2 Summary of Findings: Antivirals compared to placebo / no treatment for Infectious Mononucleosis

Patient or population: Patients diagnosed with infectious mononucleosis (glandular fever)
Setting: Hospitalised patients or out-patient setting
Intervention: Antivirals
Comparison: placebo / no treatment

Outcomes	Anticipated absolute effects* (95% CI) Risk with placebo / no treatment	Anticipated absolute effects* (95% CI) Risk with antivirals	Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
Time to clinical recovery doctor (dr) judgement	The mean time to clinical recovery dr judgement was 19.82 days	The mean time to clinical recovery dr judgement in the intervention group was 4.55 days fewer (7.67 fewer to 1.43 fewer)	-	87 (2 RCTs)	⨁⊖⊖⊖ VERY LOW123	Statistically significant reduction in favour of treatment group. Andersson 1987 had 3 patients in the treatment group that had a co-administered steroid whereas none of the placebo group has this.
Time to clinical recovery patient (pt) judgement	The mean time to clinical recovery pt judgement was 41.91 days	The mean time to clinical recovery pt judgement in the intervention group was 5.59 days fewer (26.23 fewer to 15.05 more)	-	87 (2 RCTs)	⨁⊖⊖⊖ VERY LOW123	No statistically significant difference between groups. Andersson 1987 had 3 patients in the treatment group that had a co-administered steroid whereas none of the placebo group has this.

[1] Downgraded one level due to the majority of studies included in this outcome having an unclear or high risk of bias.
[2] Downgraded one level due to differences in setting, type of antiviral, or route of medication administration.
[3] Downgraded one level due to small sample sizes or wide confidence intervals for this outcome.

Outcomes	Anticipated absolute effects* (95% CI) Risk with placebo / no treatment	Anticipated absolute effects* (95% CI) Risk with antivirals	Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
Adverse events and side effects	See comments		-	248 (5 RCTs)	⨁⊖⊖⊖ VERY LOW 134	Reported narratively only in five studies. In some reports authors were unsure whether adverse event was related to medication or complication of disease.
Time to resolution of lymphadenopathy	The mean time to resolution of lymphadenopathy was 40.52 days	The mean time to resolution of lymphadenopathy in the intervention group was 8.94 days fewer (11.75 fewer to 6.14 fewer)	-	61 (2 RCTs)	⨁⊖⊖⊖ VERY LOW 1356	Statistically significant difference in favour of treatment group. One study weighted very heavily due to high variance in other study
Development of complications of Infectious Mononucleosis	see comments		-	108 (3 RCTs)	⨁⊖⊖⊖ VERY LOW 1236	Three studies reported complications narratively. There did not seem to be any difference in the incidence of complications between treatment and control groups.
Viral shedding	see comments		-	268 (6 RCTs)	⨁⊖⊖⊖ VERY LOW 1236	Overall effect from all six studies was that viral shedding was suppressed while on antiviral

[4] Downgraded one level as no study reported adverse events as a measurable outcome.
[5] Downgraded one level as wide variance of point estimates across studies.
[6] Downgraded one level as data from one study was unpublished.

Outcomes	Anticipated absolute effects* (95% CI) Risk with placebo / no treatment	Anticipated absolute effects* (95% CI) Risk with antivirals	Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
						treatment, but this was not sustained when treatment stopped.
Days missing from school / work	The mean days missing from school / work was 20.18 days	The mean days missing from school / work in the intervention group was 0.9 days fewer (6.53 fewer to 4.74 more)	-	87 (2 RCTs)	⨁⊖⊖⊖ VERY LOW 123	No statistically significant difference between groups. Andersson 1987 had 3 patients in the treatment group that had a co-administered steroid whereas none of the placebo group has this.

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

CI: Confidence interval; RR: Risk ratio; OR: Odds ratio;

Dr: doctor, Pt: patient

GRADE Working Group grades of evidence

High quality: We are very confident that the true effect lies close to that of the estimate of the effect

Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect

Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Table 2-3 Data Analysis: Antivirals versus placebo / no treatment

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
3.1 Time to clinical recovery dr judgement	2	87	Mean Difference (95% CI)	-4.56 [-8.04, -1.08]
3.2 Time to clinical recovery pt judgement	2	87	Mean Difference (95% CI)	-5.59 [-26.23, 15.05]
3.5 Time to resolution of lymphadenopathy	2	61	Mean Difference (95% CI)	-8.94 [-11.75, -6.14]
3.6 Time to resolution of splenomegaly	2	87	Mean Difference (95% CI)	-1.68 [-5.89, 2.53]
3.7 Time to resolution of hepatomegaly	2	87	Mean Difference (95% CI)	4.75 [-9.15, 18.65]
3.8 Adverse Events and Side Effects	7		Other data	No numeric data
3.10 Development of Complications of Infectious Mononucleosis	7		Other data	No numeric data
3.13 Days missing from school / work	2	87	Mean Difference (95% CI)	-0.90 [-6.53, 4.74]
3.14 Duration of fever > 37.5deg C	2	87	Mean Difference (95% CI)	-1.52 [-6.66, 3.62]
3.15 Time to resolution of sore throat	3	117	Mean Difference (95% CI)	-1.38 [-5.68, 2.92]
3.16 Viral Shedding	7		Other data	No numeric data

CI: Confidence interval, Dr: doctor, Pt: patient

2.5.4.1 Primary Outcomes

Time to clinical recovery

Six of the seven included studies reported time to clinical recovery but reported this in different formats. There was a statistically significant reduction in favour of treatment group for this outcome as measured by physician but not by patient assessment.

Two studies reported the time to clinical recovery as a continuous outcome and subdivided this into 'patient assessment' and 'physician assessment'.(164, 168) It should be noted that in the study Andersson 1987, three patients in the treatment group had a co-administered steroid whereas none of the placebo group has this. The study Andersson 1986 reported a statistically significant reduction in time to clinical recovery as assessed by physician but not as assessed by patient. There was a much larger variation and no statistically significant difference between the two groups for either of these outcomes in Andersson 1987. When the results of the two Andersson studies were pooled for 'time to clinical recovery as assessed by physician' there was a statistically significant mean reduction of 4.56 days in the treatment group but with wide confidence intervals [-8.04, -1.08] (See Figure 2-4).(164, 168)

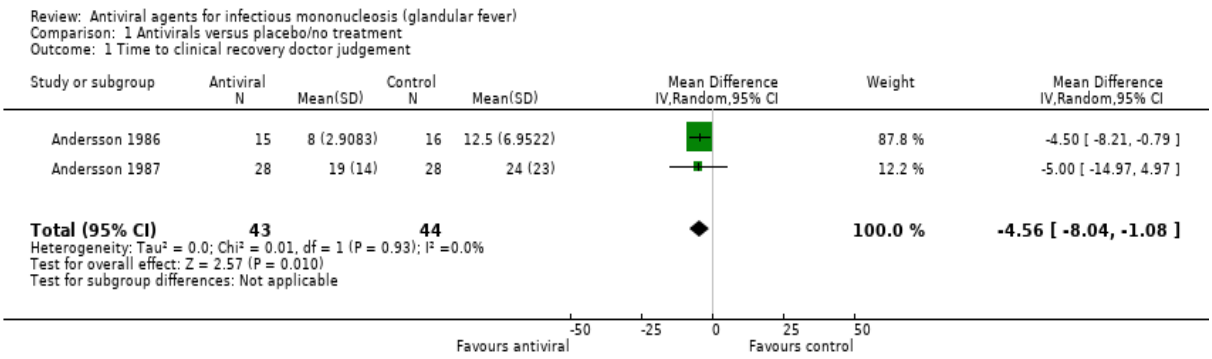

Figure 2-4 Comparison: Antivirals versus placebo/no treatment, Outcome:Time to clinical recovery doctor judgement.

Pooling the results of the two Andersson studies for the outcome ''time to clinical recovery as assessed by patient' did not yield any statistically significant result (Figure 2-5).(164, 168)

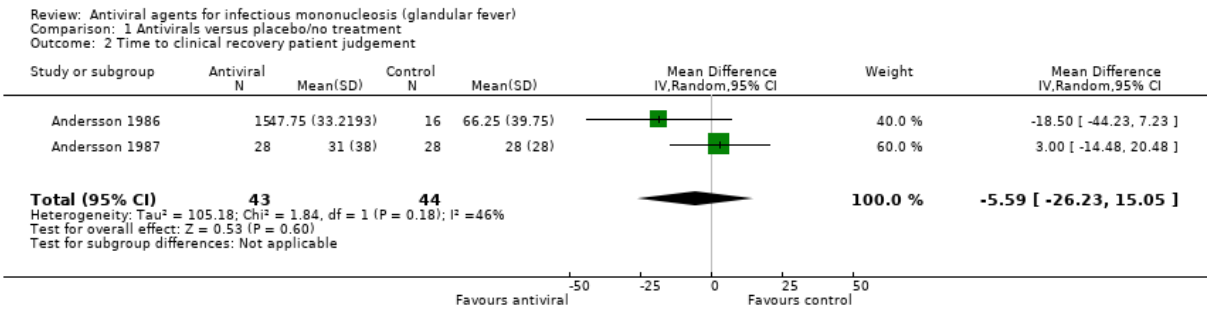

Figure 2-5 Comparison: Antivirals versus placebo/no treatment, Outcome: Time to clinical recovery patient judgement.

Van der Horst 1991 reported this as a dichotomous outcome with recovery by day 5 or day 10 with no statistically significant difference at either time point. Balfour 2007 reported the SF12 composite score at certain time points and Balfour et al 2007 and Simon 2003 reported composite scores (which were developed by the authors) at certain time points, but it was not possible to access the original data to assess these outcomes individually.

It should be noted that the data extracted from the original small studies may be skewed and it was difficult to combine in Table 2.2. Means were reported in several of the studies, however, non-parametric summaries, for example median, may be preferable for this potentially skewed data, especially if there are outliers present in the data.

Adverse events and side effects of medication

Five of the seven studies reported adverse events of medication and the majority reported no significant difference between groups for this outcome. It was not possible to pool the results for this outcome because of the potential for double counting results. Trial participants may have had more than one adverse outcome, and this was not clear in the reporting of the original studies.

Balfour et al 2007 reported that no adverse events were observed.(154)

Van der Horst 1991 reported that there was no difference between the two treatment groups in terms of side effects despite the large dose of acyclovir given.(172)

Balfour 2007 listed the adverse events in a summary format: abdominal pain, headache, nausea, vomiting, rash.(169) Of these; nausea was the only symptom where a statistically significant difference was observed between the two treatment groups with seven participants in the treatment group and one patient in the placebo group affected (p = 0.03). One serious adverse event was reported-a case of pancreatitis, but the authors stated that this was more likely to be a complication of IM rather than a side effect of medication.

Andersson 1986 reported that asymptomatic, transient elevation of serum creatinine and urea was noted in two patients from the treatment group and in none of the placebo group.(164) Thrombophlebitis was found in four patients from both treatment groups. Skin rashes were found in three of the patients treated with antiviral, but these patients were also treated with ampicillin pre-admission) and six patients from the placebo group (of whom four were given ampicillin pre-admission). During the 6-month follow-up, a total of 12 cases of upper respiratory tract infection or tonsillitis were noted. 10 of the 12 were diagnosed as bacterial complications and were evenly distributed in the two groups.

Andersson 1987 reported that three patients had an unexplained late occurrence of exanthema, one week after admission which could have been due to acyclovir or to penicillin treatment preceding enrolment in the study.(168) One patient in the acyclovir group suffered from diarrhoea and abdominal pain that resolved once acyclovir was withdrawn. No side effects were noticed in the placebo-treated patients. Serum creatinine elevation of >10% above the normal level was found in three placebo and five acyclovir-treated patients, all of which normalized within one month.

2.5.4.2 Secondary Outcomes

Time to resolution of abnormal clinical examination: (assessed by physician)

Time to resolution of fever (>37.5 degrees C)

Four of the seven included studies reported this outcome and one of these found a statistically significant difference between groups.

Simon 2003 reported this as shift in change from baseline score at day 20.(171) Van der Horst 1991 also reported a dichotomous outcome: number of patients in each treatment

group with fever at day 10.(172) There was no statistically significant difference between the two groups in either of these studies.

Both Andersson studies reported this as a continuous outcome: number of days.(164, 168) Andersson 1986 found that there was a small statistically significant reduction in this in favour of the antiviral group (median number of days four versus six in the placebo group) whereas Andersson 1987 found that there was no statistically significant difference between the two groups.(164, 168) See Figure 2-6.

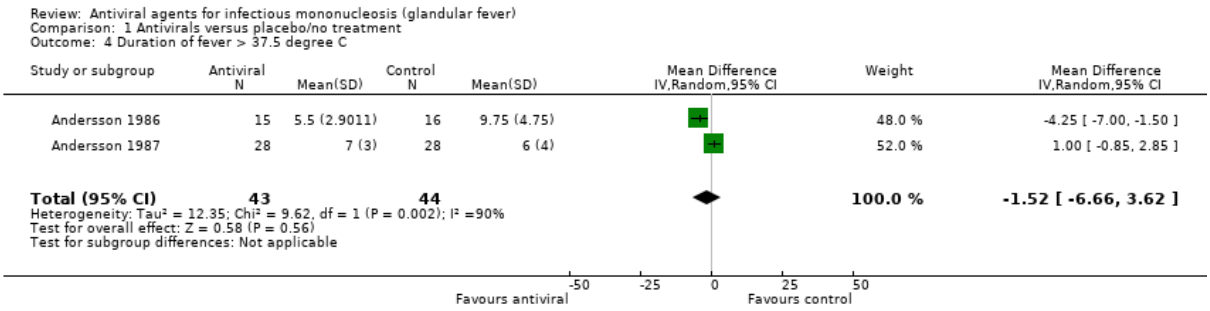

Figure 2-6 Comparison: Antivirals versus placebo/no treatment, Outcome: Duration of fever > 37.5 degree C.

Time to resolution of sore throat

Five of the seven included studies reported this outcome with one of these studies finding a significant effect in favour of antiviral treatment.

Original data obtained from the lead author of the Simon 2003 study showed a statistically significant difference between the two groups for this outcome.(171) Time to resolution in the treatment group was a mean of 6 days versus 12 in the placebo group.

Balfour et al 2007 reported the presence of sore throat for the two groups at different time points: day 1,6,10,15 (there was missing data on day 15) with no statistically significant difference between the groups at any of these time points.(154)

Both Andersson studies reported this as a continuous outcome: number of days. Both reported no statistically significant difference between the two groups.(164, 168)

Van der Horst 1991 reported a dichotomous outcome: number of patients in each treatment group with severe sore throat at day 10 with no statistically significant difference between the two groups.(172)

Pooling the results from three studies indicated no statistically significant difference between groups (MD -1.38, 95% CI -5.68 to 2.92) (Figure 2-7).(164, 168, 171)

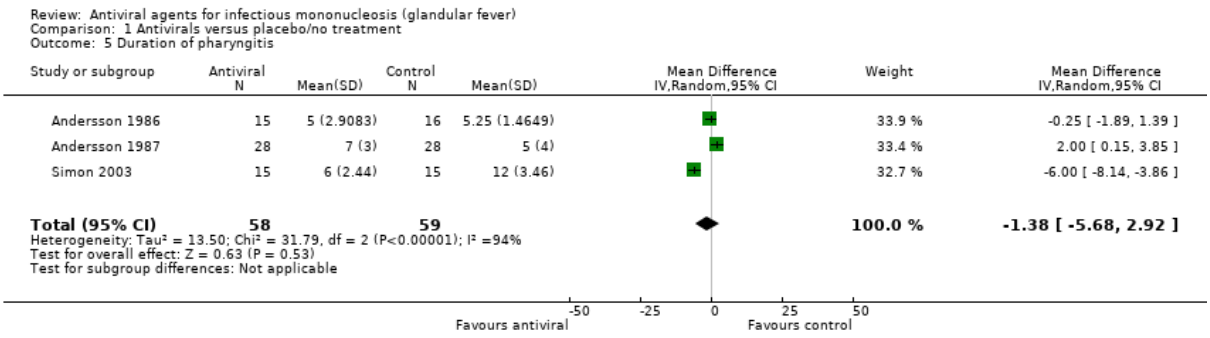

Figure 2-7 Comparison: Antivirals versus placebo/no treatment, Outcome: Duration of sore throat.

Time to resolution of lymphadenopathy

Three of the seven included studies reported this outcome, and one reported a statistically significant improvement in the anti-viral treatment group.

Original data obtained from the lead author of the Simon 2003 study showed that there was a statistically significant difference between the two groups for this outcome.(171) Time to resolution in the treatment group was a mean of 11 days versus 20 in the placebo group

The two Andersson studies reported this as a continuous outcome: number of days.(164, 168) Andersson 1986 found no statistically significant difference between the two groups.(164) Andersson 1987 reported this outcome for three patients in the treatment group who were also administered steroids.(168) It was not reported for the rest of the treatment group or the placebo group.

When the results of Andersson 1986 and Simon 2003 were pooled, there was a mean reduction in number of days of 8.94 [-11.75, -6.14] (Figure 2-8) in favour of the treatment group.(164, 171) The study Simon 2003 was weighted more than 99% in this analysis as the results for mean and SD in Andersson 1986 were so large (the original median and range

from Andersson 1986 were converted to mean and SD using the method by Hozo et al already mentioned above).(160, 171)

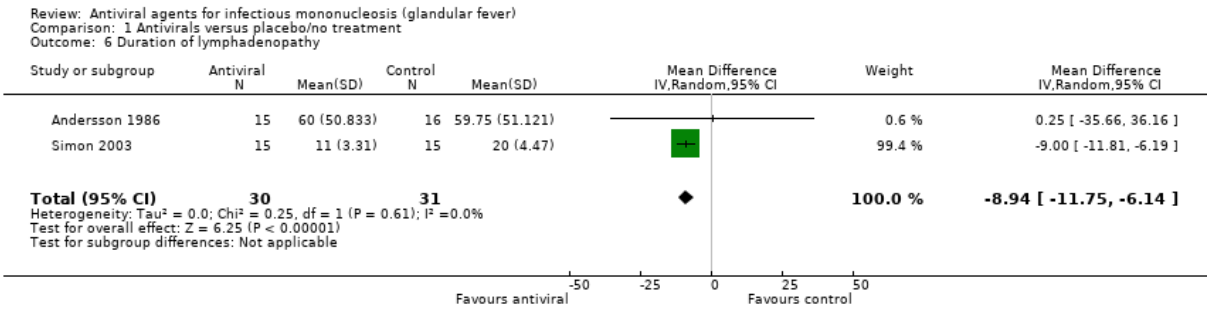

Figure 2-8 Comparison 1 Antivirals versus placebo/no treatment, Outcome 6 Duration of lymphadenopathy.

Time to resolution of splenomegaly

Two of the seven included studies reported this as a continuous outcome: number of days with no statistically significant difference between the two groups.(164, 168)

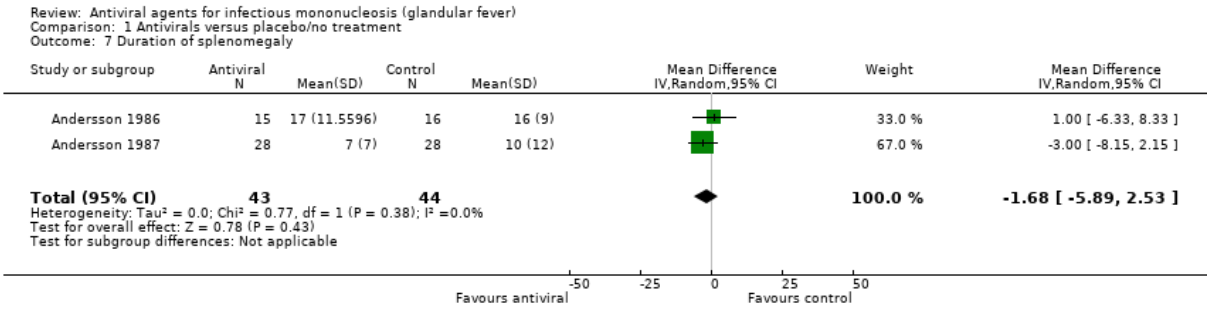

Figure 2-9 Comparison: Antivirals versus placebo/no treatment, Outcome: Duration of splenomegaly.

Time to resolution of hepatomegaly

Two of the seven included studies reported this as a continuous outcome: number of days with no statistically significant difference between the two groups.(164, 168) There was high heterogeneity in this analysis.

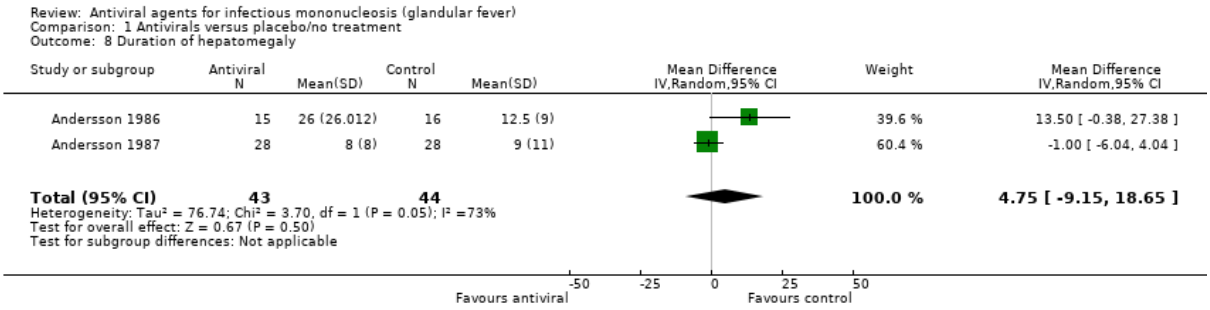

Figure 2-10 Comparison 1 Antivirals versus placebo/no treatment, Outcome 8 Duration of hepatomegaly.

Development of complications of Infectious Mononucleosis

Three of the seven included studies reported this outcome.

In Balfour 2007, a 24-year-old subject who was in the treatment arm developed acute pancreatitis on the 10th study day.(169) The authors considered that this was more likely to be a complication of infectious mononucleosis rather than an adverse drug event.

Andersson 1986 reported that one patient in the treatment group had to be tracheotomised, and acquired a transient, bilateral hypoglossal nerve palsy.(164) One patient from the placebo group was operated on 12 days after admission because of abdominal pain with a surgical diagnosis of pseudoappendicitis, and another patient in the placebo group suffered from hepatitis with mild icterus persisting for seven months, where no other aetiologic agent could be demonstrated. During the 6-month follow-up, a total of 12 cases of upper respiratory tract infection or tonsillitis were noted. 10 of the 12 were diagnosed as bacterial complications and were evenly distributed in the two groups.

Andersson 1987 reported that there were three patients in the antiviral treatment group with over-whelming clinical symptoms causing airway obstruction in two and disseminated intravascular coagulopathy in one.(168)

Viral shedding

All of the studies except for Simon 2003 reported this outcome in some way.(171) All reported similar techniques for detection of EBV oropharyngeal shedding.

Van der Horst 1991 reported inadequate sampling handling but reported the percentage of patients who were culture positive at days zero and ten.(172) The study reported that the differences between the groups were not statistically significant. It was unclear how many patients in each group these percentages were based on.

Balfour et al 2007 reported the quantity of EBV DNA in oral wash cells and supernatant at certain time points but this was only represented graphically and extrapolation from this graph or obtaining original data was not possible.(154)

Balfour 2007 reported the number of patients with ≥2log_{10} decrease in EBV copies / ml in oral cells, supernatant and whole blood at the end of treatment.(169) Of these only the data for supernatant showed a statistically significant result in favour of the antiviral, with a risk of 7.27 [95% CI 1.09, 48.35]. It also reported the median log_{10} copies EBV / ml of the oral supernatant and oral cells at certain time points, but this was only represented graphically and extrapolation from this graph was not possible. An attempt was made to obtain original data, but this was not forthcoming.

Pagano 1983 reported this as a dichotomous outcome: oropharyngeal excretion of EBV at certain time points but there are a lot of missing data here making it difficult to interpret the findings.(170)

Andersson 1986 reported the proportion of oropharyngeal EBV shedders in treatment versus placebo groups at certain time points and found a statistically significant reduction on days 4 and 7 which was not sustained at days 28 and 180 after treatment was stopped.(164)

Andersson 1987 reported the proportion of oropharyngeal EBV shedders in treatment versus placebo groups at certain time points and found a statistically significant reduction during antiviral treatment which was not sustained after treatment was stopped.(168)

Overall, each study that reported on viral shedding concluded that the antiviral drug suppressed viral shedding during treatment, but the effect was not sustained when treatment was stopped.

Patient Reported Outcome Measures (PROMs)

Five of the seven studies reported PROMs.

Health related quality of life (HRQoL)

Three of the included studies reported on HRQoL. Van der Horst 1991 reports a 'sense of well-being' in the abstract but does not actually report data for this outcome.(172)

Balfour 2007 reports results for the SF12 composite score which was examined at day 1 and day 28. There was no statistically significant difference between treatment and placebo groups.(169)

Simon 2003 reported a composite score ('feeling bad score', scored as absent (0), mild (1), moderate (2), or severe (3) from a scoring system developed by the authors) which found a non-significant shift in change from baseline at day 20 between treatment and placebo groups.(171)

Days missing from school of work

Four of the seven included studies reported on days missing from school or work with data available only for two of the studies which found no difference between groups.

Simon 2003 referred to a 'pattern of activities' and Balfour 2007 reported composite scores but it was not possible to obtain original data for these outcomes.(169, 171)

Both Andersson studies reported this as a continuous outcome: number of days with no statistically significant difference between the two groups.(164, 168) Pooling these two trial results did not give any statistically significant result either (MD -0.90, 95% CI -6.53 to 4.74) (Figure 2-11). There was moderate heterogeneity in this analysis.

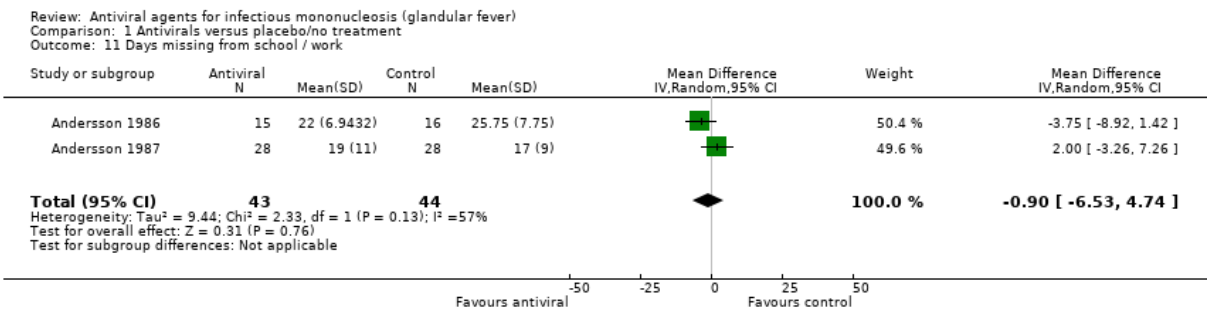

Figure 2-11 Comparison 1 Antivirals versus placebo/no treatment, Outcome 11 Days missing from school / work.

Economic Outcomes

Economic outcomes were not mentioned in any of the studies.

2.5.4.3 Subgroups

It was planned to undertake subgroup analyses based on patient age, setting and placebo versus no treatment controls with sufficient data but this was not possible due to lack of appropriate data.

2.6 Discussion

2.6.1 Summary of main results

Results of this review need to be interpreted with caution as the quality of the evidence was graded as very low for all outcomes. There was a statistically significant improvement in the treatment group for only two of the twelve outcomes reported in two out of the seven included studies. Both of these outcomes were physician assessed outcomes and may have limited clinical importance.

Pooled results for 'Time to clinical recovery as assessed by physician' indicated a mean reduction in number of days of 4.56 days in the treatment group with wide confidence intervals [-8.04, -1.08]. However, it was unclear how this outcome was assessed in the studies reporting it and considering that prospective studies report that clinical signs and symptoms start to resolve by one month ((26); (27)) and that fatigue may be persistent in approximately ten percent of patients at six month follow up ((32); (62)) this may not be a clinically meaningful result. In the trial Andersson 1987, three of the treatment group had steroids as a co-intervention while none of the placebo group did which may have affected the results also.(168)

Trial results for the outcome 'adverse events and side effects of medication' could not be pooled due to the potential for double counting results but overall, the majority of trials reporting this outcome did not find any significant difference between treatment and control groups.

Results from were pooled for two studies for the outcome 'Time to resolution of lymphadenopathy', with a mean reduction in number of days of 8.94 [-11.75, -6.14] in

favour of the treatment group.(164, 171) Within this meta-analysis the study Simon 2003 was weighted more than 99%, as the standard deviations in Andersson 1986 were so large. These two trials also reported very heterogenous results for this outcome, with Andersson 1986 reporting lymphadenopathy of more than 1 cm diameter, whereas Simon 2003 reported the presence of lymphadenopathy as determined by physician assessment, but it is not clear what the criteria for reporting presence or absence was.(164, 171)

The overall effect on viral shedding from the six studies that reported this outcome was that viral shedding was suppressed while on antiviral treatment, but this effect was not sustained when treatment was stopped.

For the eight other outcomes reported in included studies there was no statistically significant effect of anti-viral therapy in IM.

2.6.2 Overall completeness and applicability of evidence

Seven trials with a total of 333 participants met the inclusion criteria for this review. The trials were published between 1983 and 2007. In general, the number of participants in each trial was small; only one trial had more than 60 participants.(172) Also, many of the effect sizes had wide confidence intervals relating to the small sample sizes, reducing the precision of estimates. It is also possible that these studies were underpowered to detect potential differences in many of the secondary outcomes due to the small sample sizes. The trials were heterogenous in terms of setting (out-patient v's inpatient), severity of illness, antiviral treatment regimens (differences in antiviral used, dose and method of administration), age of participants (children v's young adults) outcomes assessed and reporting of data. Pooling of results was limited as a result of this heterogeneity, with results from three trials or less being pooled for any one outcome. It was not possible to perform subgroup or sensitivity analyses for any of the outcomes. All included trials had inadequate outcome reporting. All included trials had either an unclear or high risk of bias. None of the trials reported on economic outcomes. These factors all limit the conclusions that can be drawn.

Two ongoing trials were found in the updated December 2021 search.(166, 167) Both of these trials included children only, but inclusion criteria and outcomes differed slightly

between the two trials. It would be important to include these studies in an updated review in the future.

2.6.3 Quality of the evidence

The quality of the evidence was graded as very low for all outcomes which means that 'the true effect is likely to be substantially different from the estimate of effect' as per the GRADE Working Group grades of evidence. The evidence was downgraded in the domains of risk of bias due to the majority of studies having an unclear or high risk of bias; indirectness due to due to differences in setting, type of antiviral, or route of medication administration; imprecision due to small sample sizes or wide confidence interval; inconsistency due to wide variance of point estimates across studies; and publication bias due to one of the seven included studies being unpublished.(169)

Overall, the very low quality of the evidence means that very little confidence can be placed in the results found.

2.6.4 Potential biases in the review process

Every effort was made to limit bias in the review process. Though a thorough search strategy was used, it's possible that all trials eligible for inclusion were not identified, especially unpublished trials. Publication bias is a possibility because the majority of the included trials in this review were supported by a pharmaceutical company. It is known that the risk of publication bias is probably higher for reviews that are based on industry funded trials (173).

2.6.5 Agreements and disagreements with other studies or reviews

Although the review included two additional trials, the results are in agreement with the previous 1999 systematic review, concluding that there is not enough evidence to support the use of antiviral agents for IM.(52) A recent narrative review article also concluded that while treatment with acyclovir significantly decreased the rate of oropharyngeal viral shedding, there was no evidence to support its use in an acute clinical setting.(25)

Studies examining the effect of antivirals for other acute illnesses have generally found small improvements in outcomes in favour of antivirals but with the potential negative effects of medication costs, side effects and potential for antiviral resistance. A 2015 meta-analysis published in the Lancet found that in adults with a laboratory diagnosis of influenza, oseltamivir accelerated time to clinical symptom alleviation, reduced the risk of antibiotic prescribing for lower respiratory tract infection and hospital admission for any cause.(174) There was no benefit conferred to patients who had symptoms of influenza like illness but not confirmed infection. A 2015 Cochrane review of Antivirals for Bell's Palsy concluded that there was a benefit from the combination of antivirals with corticosteroids compared to corticosteroids alone for the treatment of Bell's palsy of various degrees of severity but this was based on low-quality evidence.(175) A Cochrane review of Acyclovir for Varicella found that acyclovir was effective in reducing the number of days with fever and the maximum number of lesions but didn't have an effect on complications or relief of itch among otherwise healthy children with chickenpox.(176)

2.7 Authors' conclusions

2.7.1 Implications for practice

This review confirms that there is currently no evidence base for prescribing antiviral agents in acute IM; the majority of included studies were of sub optimal quality at unclear or high risk of bias and so questions remain about the effectiveness of this intervention. Although two of the outcomes have results that favour treatment over control, the quality of the evidence that these results is based on is very low and they represent a minority of outcomes reported in this review. Along with lack of effectiveness, there are associated negative consequences of costs, potential adverse events and antiviral resistance.

2.7.2 Implications for research

Preventing morbidity associated with infectious mononucleosis is a priority for health systems. However, the current evidence in this area is limited in respect to not only antiviral medication but also in terms of other interventions.

In 2015, an updated Cochrane review evaluating the effectiveness of corticosteroids for the control of symptoms of IM concluded that there was insufficient evidence of clinical benefit.(53)

Also, Metronidazole, an anaerobic antibacterial agent, has been studied in severe cases of patients with IM who were hospitalised and found to reduce hospital stay in a study of 42 patients, but many of the other studies done in this area are from the 1980's.(55)

More robust clinical trials are required to further assess this research question as the quality of the current evidence is poor and based on small heterogenous studies. Most cases of symptomatic IM are encountered in young adults in a primary care environment, often in student health centres. Trials of commonly used orally administered antivirals versus usual care should be established in this setting. Outcomes examined should include effectiveness on acute symptoms, adverse effects, time off work or school, effect of antivirals on longer term outcomes such as fatigue, and evaluation of economic outcomes.

3 Chapter 3: Cohort study set-up and characteristics

3.1 Cohort study establishment and follow-up

This cohort study was established in 2017 with aims as described in Section 1.8.

The Strengthening and Reporting of Observational Studies in Epidemiology (STROBE) and Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) guidelines were utilised in reporting this methodology section and a summaries are presented in Appendix 1: STROBE checklist for prospective cohort studies, Appendix 2: TRIPOD checklist: prediction model development, Appendix 3: TRIPOD checklist: prediction model validation (EBV CPR score)and Appendix 4: TRIPOD checklist: prediction model validation (Centor score).

3.2 Study design

The study was a prospective cohort study, with follow up at three and six months. Ethical approval was initially granted for this study from the Royal College of Surgeons in Ireland (RCSI) research ethics committee in 2017. An extension of ethical approval, to allow adequate time for follow-up data collection to occur (Reference number: REC201908006, see Appendix 5: Ethical approval and amendments for cohort study), was granted in 2019.

Patients consented at baseline for a blood sample and a throat swab to be taken to be used by the research team for analysis, to provide information to the research team through questionnaires, to being contacted by the research team for the purposes of study follow up and allowed the research team to access their clinical records in relation to data collected during the study.

3.2.1 Setting

The study was set in primary care student health centres in seven third level institutions in Ireland. The health centres were distributed as follows: two in Dublin (Dublin Institute of

Technology (DIT) and Trinity College Dublin (TCD)), two in Cork (University College Cork (UCC) and Cork Institute of Technology (CIT)), one in Galway (National University of Ireland Galway (NUIG)), one in Limerick (University of Limerick (UL)) and one in Maynooth, Co. Kildare (Maynooth University (MU)). Participating GPs were awarded continuing professional development points, along with an audit template for their involvement. Recruitment took place from September 2017 until May 2019. Recruitment was stopped temporarily in May 2019, as the PhD candidate was taking maternity leave, and it was planned that recruitment would re-commence on return from maternity leave in September 2020, however, the Covid-19 pandemic meant that recruitment could not be recommenced.

3.2.2 Participants

3.2.2.1 Eligibility criteria

Potential participants were required to meet the following criteria, to be considered eligible for this study;

- aged over 18 with no upper age limit
- presenting with sore throat and at least one other of the following symptoms: malaise, fatigue, lymphadenopathy, fever, headache
- symptom duration 7 days or less
- willing and able to give informed consent and comply with all trial requirements

Participants were excluded if they met any of the following exclusion criteria as determined by their GP:

- condition or treatment associated with significant impaired immunity
- health literacy or language difficulties which means that the study information was not easily understood

3.2.3 Process of data collection

Participants were given written information (Appendix 6: Participant Information Sheet, Appendix 7: Participant Information Sheet, short version) about the study before deciding

whether to sign a consent form (Appendix 8: Participant Consent Form) to participate. Each participant had a participant questionnaire (Appendix 9: Participant Questionnaire) and case report form (CRF) (Appendix 10: Case Report Form (CRF)) completed at recruitment detailing their symptoms and examination signs. The recruiting clinician (either GP or practice nurse) collected data in the case report form, and this combined with the data collected in the participant questionnaire to give information about possible explanatory variables.

Follow-up data was collected at three and six month time points. The PhD candidate initially emailed the participant to collect this data via a link to an online survey. In order to minimise participants being lost to follow up, if the participant did not submit their follow up questionnaire, they were followed up with a further email. If they still did not submit their questionnaire, they were followed up with a text message reminder.

3.2.4 Variables

The 28 explanatory variables were chosen based on a systematic review of signs and symptoms for the diagnosis of infectious mononucleosis along with further variables based on consensus between clinical colleagues, including GPs involved in clinical and academic general practice.(40, 177) The possible explanatory variables collected at baseline were:

1) Age at recruitment
2) Gender
3) Cough*
4) Nasal congestion*
5) Shortness of breath*
6) Subjective history of fever*
7) Headache*
8) Muscle aches / pains*
9) Sweats / chills*
10) Fatigue*
11) Not sleeping well*

12) Dizziness*

13) Feeling generally unwell*

14) Poor appetite*

15) Objective temperature ≥ 38 degrees Celsius

16) Pulse

17) Respiratory rate

18) Exudate on pharynx

19) Exudate on tonsils

20) Palatal petechiae

21) Enlarged anterior cervical lymph nodes

22) Tender anterior cervical lymph nodes

23) Enlarged / tender posterior cervical lymph nodes

24) Enlarged / tender inguinal lymph nodes

25) Enlarged / tender axillary lymph nodes

26) Splenomegaly

27) Hepatomegaly

28) Atypical lymphocytosis >10% (dichotomous outcome as reported on FBC result)

*= dichotomous outcomes reported by participant at baseline

The participant questionnaire included a baseline measure of fatigue using the Fatigue Severity Scale (FSS) and an unvalidated simple visual analogue scale (VAS) for global fatigue. The FSS is a validated instrument for measuring fatigue. A 2009 systematic review examining the performance of fatigue measures concluded that 'there is no ideal instrument' but that the FSS demonstrates good psychometric properties and demonstrates the ability to detect change over time.(64) Another review of measures of fatigue found that the FSS had excellent reliability and validity.(65) It has been validated in populations with chronic fatigue syndrome but not specifically in populations with fatigue post infectious mononucleosis.
The recruiting clinician sent two blood samples to the local laboratory, as part of routine diagnostic testing. All of the laboratories used are attached to public general hospitals local to the recruiting sites. One sample was analysed for EBV serology, and the other had a full blood count (FBC) performed to check for lymphocytosis. The recruiting clinician also sent a

throat swab to their local laboratory to be cultured and tested for Group A Beta Haemolytic Streptococcus (GABHS).

The participant's GP managed the patient as usual, which generally involved symptomatic treatment for suspected infectious mononucleosis or appropriate management of the participant's medical needs if another diagnosis was suspected.

3.2.5 Outcome Measures

The outcome measure for the EBV CPR derivation study was the diagnosis of acute Infectious Mononucleosis caused by EBV. The gold standard reference test for this was positive EBV serology, more specifically positive anti-VCA (viral capsid antigen) IgM (Immunoglobulin M). The EBV serology was analysed in two ways, depending on the local laboratory involved. One of the laboratories (which analysed the majority of participants samples) screened initially for EBNA (EBV nuclear antigen)-1 IgG (Immunoglobulin G). EBNA IgG becomes positive in the resolution phased of IM and remains positive for life. If EBNA IgG was positive, the EBV infection was unlikely to be recent (> at least 4 weeks ago); if EBNA-1 IgG was negative then testing was performed for ani-VCA IgM and anti-VCA IgG. Other laboratories test for anti-VCA IgM and anti-VCA IgG initially on the basis that EBNA-1 IgG may be positive early in infection, but this is very infrequent. Both methods result in the anti-VCA IgM being positive for a positive test result. Specificity and sensitivity for anti-VCA IgG and IgM antibodies are approximately 94% and 97% respectively.(45)

The outcome measure for the EBV CPR validation study the was the diagnosis of acute Infectious Mononucleosis caused by EBV. The reference standard test in the derivation population was EBV serology, which differed from the external validation population, where a heterophile antibody test was used for diagnosis. Ideally, the diagnostic tests in the derivation and validation populations would be the same, however, this was not possible in this study.

The outcome measure for the Centor score CPR validation was the diagnosis of GABHS sore throat. The reference standard for diagnosis of GABHS was by throat swab culture, despite several drawbacks, see also Section 1.3.3.(77)

Two main outcomes were examined in the observational cohort study; Time to return to college / work in number of days, and two measures of fatigue. This data was collected at three and six month follow up.

3.2.6 Sample Size

3.2.6.1 EBV CPR derivation study

Based on information received from one of the main Dublin hospital laboratories (personal communication: Dr B. Crowley, consultant microbiologist, St. James Hospital), approximately one third of tests performed for EBV IM have a positive result. Allowing that there was a lower threshold for testing in this study, and more people were being tested than usual, for the purposes of calculating the sample size a conservative estimate of the ratio of cases to non-cases of 1:4 was used rather than this 1:2 ratio.

The guidance for estimating the sample size required to derive a CPR advises a minimum of 10 participants with the outcome and 10 participants without the outcome for each explanatory variable used.(178) To estimate the sample size for the derivation of the CPR the four most likely clinical variables, along with a further two variables; tonsillar exudate and objective fever, based on consensus between clinical colleagues, were chosen. Using these six variables a sample size of 300 participants (with sore throat at presentation) was calculated. Data was also collected on several additional clinical variables, to reduce the risk of missing a variable that may have proved predictive. Unfortunately, for two main reasons the target sample size for the number of cases required was not reached (although the target for the number of controls was reached); firstly, the ratio of cases to non-cases was approximately 1:7 (rather than the predicted 1:4), resulting in much fewer cases than anticipated, and, secondly, due to the Covid-19 pandemic, recruitment for the study was ceased prematurely.

3.2.6.2 EBV CPR validation study

There is no agreed consensus on determining an adequate sample size in external validation studies (Moons et al., 2015), however, a minimum of 100 events and 100 non-events is recognised as an acceptable sample size when externally validating a CPR.(179)

From the literature, EBV IM accounts for approximately 8% of cases of sore throat. However, the validation population differed, in that it consisted of patients in whom IM was suspected and the treating clinician requested a diagnostic test. As previously mentioned, approximately one third of tests performed for EBV IM have a positive result. Using this 1:2 ratio, for the purposes of calculating sample size, it was estimated that a sample size of approximately 300 would be required to yield 100 events of EBV IM.

3.2.6.3 Centor score validation study

As mentioned in the previous section, usually a minimum of 100 events and 100 non-events is accepted as an adequate sample size when externally validating a CPR.(179)

From the literature, GABHS accounts for up to 15% of cases of sore throat, so it was estimated that a sample size of approximately 667 would be required to yield 100 events of GABHS (although the prevalence of GABHS on throat swabs in the original Centor score derivation study was 26%).(74) However, target sample size was not reached for this study either.

3.3 Risk of bias

Bias may be introduced in a number of areas in this cohort study; however, efforts have been made to minimise this as much as possible.

Bias may have been introduced to participants and recruiting clinicians as the study was introduced to them as being focussed on sore throat, and in particular, infectious mononucleosis. Although inclusion criteria were broad (sore throat and one other of malaise, fatigue, lymphadenopathy, fever or headache), recruiting clinicians may have selected cases for inclusion more on the basis that they felt that the participant may have had IM. However, the recruiting clinicians were not involved in the development of the CPR and were not briefed on the existing literature in this area, so it was assumed that assessment of predictors by the clinicians was done in an unbiased manner.

There may be confirmation bias on the part of the recruiting clinician, or participant who self-reports their symptoms, as much of the case report form and participant questionnaire is in the 'tick box' format.

Assessment for the main diagnostic outcome, i.e., testing for EBV serology, would not have been subject to bias, as this is done in a laboratory setting, separate from the clinical recruitment setting.

Participants did not know their diagnosis at baseline, when they had measures taken for fatigue scores. Knowing their diagnosis at three and six month follow up may potentially bias them into reporting higher scores. However, it would not have been possible to blind patients to their diagnosis.

3.4 Characteristics of cohort population

3.4.1 Recruitment of participants

A total of 348 participants were recruited from student health centres in the third level institutions already mentioned. 219 participants were recruited from NUIG, 55 from UL, 34 from UCC, 22 from TCD, 11 from CIT, 6 from DIT and 1 from MU. The proportion of participants who tested positive for EBV varied between the sites and may be accounted for by differences in recruitment methods, although all sites should have used the same inclusion criteria (Table 3-1). It is likely that recruiting clinicians in certain sites may have

focussed more on suspected cases of IM for study inclusion, rather than adhering strictly to the broad inclusion criteria. This is a limitation of the study and may have impacted on the results, in terms of the prior probability of the diagnosis being higher in sites where recruitment was more selective for those with features suggestive of IM, rather than just the inclusion criteria.

Table 3-1: proportion of participants recruited from different sites

Recruitment Site	NUIG	UL	UCC	TCD	CIT	DIT	MU
Number recruited at site	219	55	34	22	11	6	1
% recruited at site	62.9	15.8	9.8	6.3	3.2	1.7	0.3
% who were positive for EBV at each site	22/219 10.0	4/55 7.3	10/34 29.4	3/22 13.6	2/11 18.2	1/6 16.7	0/1 0

3.4.2 Demographics of participants

There were 225 females, 122 males and one gender neutral person recruited. The gender variable from the gender-neutral person was omitted from analysis, as any associations found in relation to just one participant would not lead to accurate conclusions. However, all other data from this participant was used.

The participants ages ranged from 17-39 years old, with the mean being 20.6 years old. Mean age for the group that were EBV positive was 20.7, while the mean age for the group that were GABHS positive was 21.0.

All of the participants recruited were within a narrow age range, and all were attending third level education in a developed country. This limits the generalisability of the results in different settings; for example, a broader age range and those living in areas of social deprivation or developing countries.

3.4.3 Diagnoses of participants in study cohort

There were missing results / non reference standard results (i.e., equivocal results / positive and negative Monospot tests) for EBV serology (n=20) and missing results for throat swab (n=6). Despite written and verbal briefing in the study recruitment methods, one of the recruitment sites initially used Monospot test rather than EBV serology, and these results had to be excluded.

Number of participants with positive serology result for EBV:	42 / 328 (12.8%)
Number of participants with positive swab result for GABHS:	80 / 342 (23.4%)
Number of participants with negative results for both GABHS and EBV:	205 / 327 (62.7%)
Number of participants with positive results for both GABHS and EBV:	5 / 327 (1.5%)
Number of participants with positive result for GABHS, Streptococcus C/G or Fusobacterium Necrophorum:	118 / 342 (34.5%)

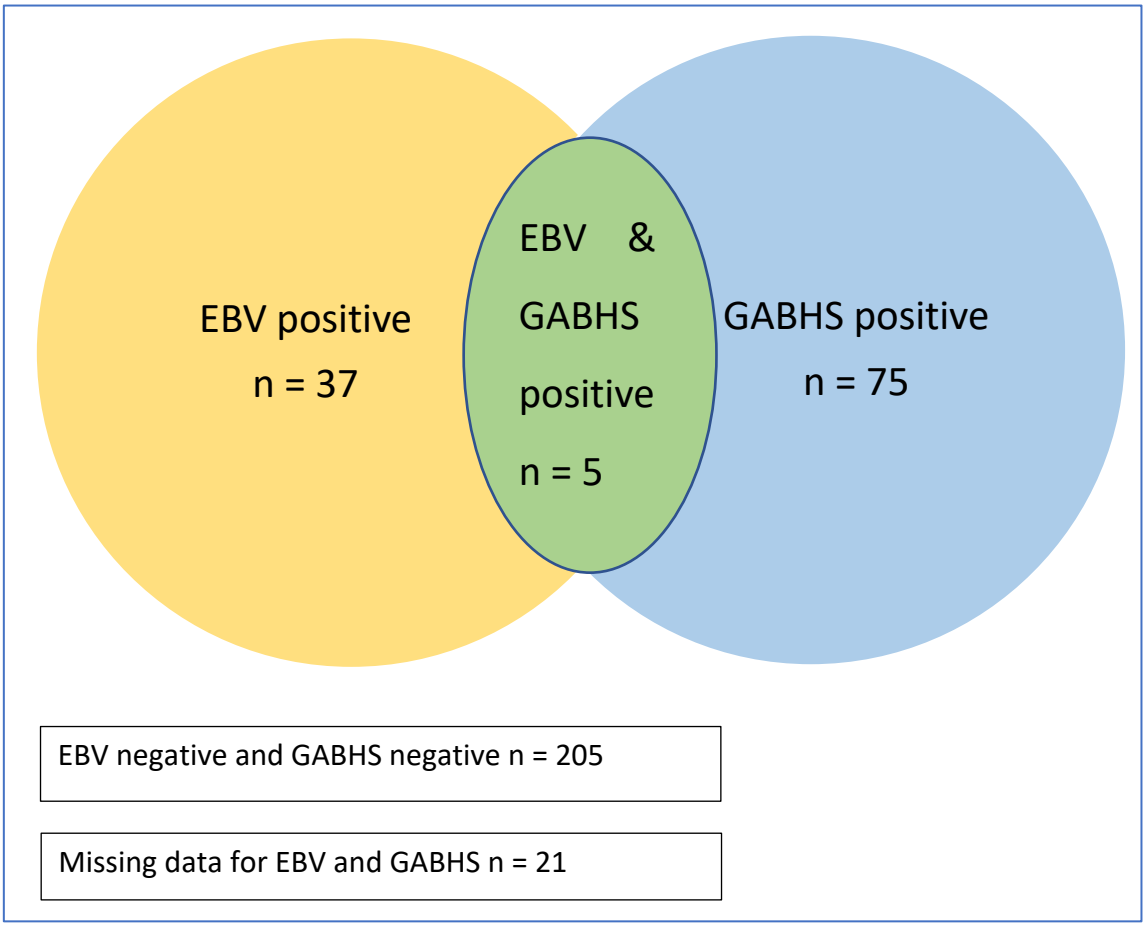

Figure 3-1: Number of participants with positive / negative / missing results for each condition

Our cohort differs from other cohort studies in that we have studied two different conditions, in a young student health population.

An Australian study from 1995 examining incidence of various respiratory illnesses in sentinel general practices, found that the annual incidence of IM was highest in the age range 16-20 years, accounting for 7.9% of cases of sore throat in this group.(6) The rate was 2.1% in those aged 5-15 years, 3.2% in the 21-25 years age range, and 1.7% in the 26-35 years age range. For people presenting with sore throat in all other age groups 1% or less were diagnosed as having IM. Our cohort population (with a mean age of 20.6 years old) had a rate of EBV positivity of 12.8% which is higher than reported in the Australian study. That incidence study is now more than 25 years old, and our higher rate may reflect differences in the testing methods or assays used, or differences in the study population (i.e., standard primary care versus student health primary care).

A systematic review from 2000 examining the signs and symptoms that were most predictive of sore throat, found rates of between 10-36% of GABHS in adults and children presenting with sore throat.(9) In the two large studies in the systematic review that included only adults, the rates of GABHS were 10-15%.(3, 7) A UK study from 2014, which used two cohorts (both including adults and children) in a clinical diagnostic study, found rates of GABHS of 31% and 23% in their cohorts.(180) The rate of positivity for GABHS in our study population was 23.4%. This is higher than the rates found in previous studies, and may represent an element of carriage of GABHS, which is known to be higher in younger adults and children (see also Section 1.3.2).

Another explanation is that the higher rate of both EBV and GABHS in the study population is influenced by the inclusion criteria; which were sore throat and one other of the following symptoms: malaise, fatigue, lymphadenopathy, fever, headache. A combination of sore throat with one of these symptoms very likely made a diagnosis of EBV IM and GABHS more likely.

The proportion of participants who had positive test results for both EBV and GABHS was 1.5%. These almost certainly were cases where their acute symptoms were caused by EBV, but they had a positive throat swab for GABHS due to carrier status. There were likely to be many more participants who had positive throat swabs for GABHS, but whose symptoms

were caused by acute viral illness rather than acute GABHS. It is impossible to quantify this figure, however, as testing for all viral causes of sore throat is not possible.

3.4.4 Symptoms and signs of participants with EBV and GABHS

Table 3-2 demonstrates a summary of individual symptoms or signs and positivity for EBV and GABHS. The total number of participants with results for EBV was 328, and the total number of participants with results for GABHS was 342. The case report form (to be completed by clinician) only included a tick box for present or absent, and so for signs where absent was ticked, this was taken to mean 'examined for and sign not identified'. Where neither box was ticked, this was recorded as incomplete data. There was more incomplete data for signs like splenomegaly and enlarged / tender inguinal lymph nodes, which are more invasive to examine for. However, in some cases, clinicians may have ticked the box for a variable being absent, when in fact it wasn't examined for.

Table 3-2: Summary of variables for EBV and GABHS positivity

Symptom / sign	EBV positive n=42 N (%)[a]	EBV negative n=286 N (%)[a]	GABHS positive n=80 N (%)[a]	GABHS negative n=262 N (%)[a]
Temperature >38	2 (6.5)	9 (3.6)	3 (4.3)	8 (3.6)
Exudate on tonsils	25 (61.0)	137 (48.2)	44 (55.0)	125 (48.1)
Splenomegaly	2 (5.1)	1 (0.4)	0	3 (1.2)
Palatal petechiae	11 (27.5)	81 (28.6)	26 (32.9)	67 (25.9)
Enlarged / tender posterior cervical lymph nodes	22 (52.4)	54 (19.0)	16 (20)	60 (22.9)
Enlarged / tender inguinal lymph nodes	2 (5.1)	13 (4.9)	3 (4.3)	12 (4.9)
Enlarged / tender axillary lymph nodes	2 (5.1)	9 (3.5)	2 (2.8)	9 (3.8)
Gender [b] **Males** **Females**	 15 (35.7) 26 (61.9)	 104 (36.4) 182 (63.6)	 24 (30.0) 56 (70.0)	 94 (36.0) 167 (64.0)
Cough	18 (43.9)	130 (45.8)	25 (31.7)	124 (48.1)
Nasal congestion	21 (51.2)	164 (57.8)	36 (45.6)	158 (60.8)
Shortness of breath	7 (17.1)	83 (29.3)	18 (22.8)	75 (29.1)
Feeling generally unwell	33 (80.5)	252 (88.7)	70 (88.6)	228 (87.7)
History of fever	28 (68.3)	170 (60.5)	45 (57.0)	163 (63.4)
Headache	27 (65.9)	165 (58.1)	50 (63.3)	155 (59.6)
Muscle aches	21 (51.2)	142 (50.4)	45 (57.0)	128 (49.8)
Sweats / chills	28 (68.3)	189 (66.8)	59 (74.7)	167 (64.7)
Fatigue	36 (87.8)	230 (81.6)	67 (84.8)	211 (81.8)

Symptom / sign	EBV positive n=42 N (%)[a]	EBV negative n=286 N (%)[a]	GABHS positive n=80 N (%)[a]	GABHS negative n=262 N (%)[a]
Not sleeping well	28 (68.3)	200 (70.7)	57 (72.2)	178 (68.7)
Dizziness	10 (25.0)	93 (32.8)	26 (32.9)	80 (31.0)
Poor appetite	22 (53.7)	174 (61.3)	48 (60.8)	156 (60.0)
Exudate on pharynx	18 (43.9)	59 (20.7)	19 (23.8)	59 (22.6)
Enlarged anterior cervical lymph nodes	37 (88.1)	241 (84.6)	72 (90)	219 (83.6)
Tender anterior cervical lymph nodes	33 (78.6)	208 (73.0)	68 (85.0)	184 (70.2)
Hepatomegaly	0	4 (1.5)	0	4 (1.5)
Respiratory rate 0-11	2 (7.7)	4 (2.0)	1 (1.7)	4 (2.2)
Respiratory rate 12-16	21 (80.8)	144 (70.2)	39 (65.0)	138 (74.6)
Respiratory rate 17-28	3 (11.5)	57 (27.8)	20 (33.3)	43 (23.2)
Atypical Lymphocytosis >10%	26 (63.4)	3 (1.1)	2 (2.6)	26 (10.0)
Clinician impression EBV positive	12 (28.6)	17 (5.9)	N/A	N/A
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age	20.69 (3.94)	20.56 (2.63)	21.01 (3.69)	20.44 (2.45)
Pulse	82.82 (13.92)	81.08 (10.99)	81.62 (11.24)	81.12 (11.42)

[a] Calculations exclude those with missing data for each variable.

[b] The gender variable from the gender-neutral person was omitted from analysis, as any associations found in relation to just one participant would not lead to accurate conclusions. However, all other data from this participant was used.

3.4.5 Response rates of participants

Of the 348 participants recruited, 239 (68.7%) submitted compete follow up data at three months, and 196 (56.3%) submitted complete follow up data at six months. The response rates were slightly higher for EBV positive when compared to EBV negative participants (Table 3-3). Table 3-3 demonstrates the response rates for the fatigue scores follow up data at three and six months, and also days off work / college reported at three months (overall proportions for EBV positive and negative at baseline were 12.8% and 87.2% respectively).

Table 3-3: Follow up rates by EBV positivity

Response received at follow up	EBV positive (n=42) n (%)	EBV negative (n=286) n (%)
FSS 3 month follow up	31 (73.8)	193 (67.5)
FSS 6 month follow up	25 (59.5)	163 (57.0)
VAS for fatigue 3 month follow up	31 (73.8)	191 (66.8)
VAS for fatigue 6 month follow up	25 (59.5)	162 (56.6)
Days off work / college (reported at 3 months)	36 (85.7)	223 (78.0)

FSS= fatigue severity scale
VAS= visual analogue scale

4 Chapter 4: Derivation of a clinical prediction rule to aid in the diagnosis of IM

4.1 Introduction

4.1.1 Background

IM is primarily a disease of adolescents and young adults. An Australian study from 1995 examining incidence of various respiratory illnesses in sentinel general practices, found that the annual incidence of IM was highest in the age range 16-20 years, accounting for 7.9% of cases of sore throat in this group.(6) The rate was 2.1% in those aged 5-15 years, 3.2% in the 21-25 years age range, and 1.7% in the 26-35 years age range. For people presenting with sore throat in all other age groups 1% or less were diagnosed as having IM.

4.1.2 Diagnosis

The diagnosis of IM is usually a clinical one, based on characteristic features, but there are no agreed clinical diagnostic criteria. Laboratory tests can be used to confirm the diagnosis where there is clinical suspicion. The gold standard reference test for EBV IM is EBV serology. This is discussed in more detail in Section 1.2.5.

4.1.3 Clinical prediction rules

As previously described in Section 1.6.3, 'clinical prediction rules' (CPRs) or 'clinical decision rules' are clinical aids that measure the independent impact of factors from a patient's history, clinical examination and diagnostic tests, and classify patients according to the probability of having a disorder of interest.(121)

There are no widely used CPRs for IM. A 2021 systematic review of diagnostic accuracy of clinical signs, symptoms and haematological parameters for IM included 17 studies from 1977-2013.(40) The included studies reported sufficient data to calculate the diagnostic accuracy of clinical signs or symptoms or white blood cell count, in participants with sore throat or those in whom their clinician suspected a diagnosis of IM. The review concluded that the presence of posterior cervical lymphadenopathy, inguinal or axillary

lymphadenopathy, palatine petechiae, splenomegaly or atypical lymphocytosis +/- high lymphocyte count increased the likelihood of a diagnosis of IM. However, most of the included studies did not identify the severity or duration of participants' symptoms, and some of the studies used typical symptoms of IM (e.g. sore throat) as their inclusion criteria, so the estimation of the diagnostic accuracy of such symptoms and signs might have been biased. The review concluded that well-designed prospective studies are required to further examine the importance of clinical and lab based findings and study the natural history of IM.

4.2 Aims and objectives

The aim of this study was to develop a CPR to aid in the clinical diagnosis of IM caused by EBV.

The rationale for developing a CPR for the diagnosis of IM is to; facilitate early positive diagnosis of IM, avoid unnecessary testing in low-risk cases, guide symptomatic management and advice for patients (avoiding potential complications e.g., splenic rupture if patient is not aware of their diagnosis), to give a more accurate prognosis, and to avoid unnecessary side effects from the inappropriate use of antibiotics in a viral illness.

4.3 Methodology

A detailed description of the cohort study design, setting, participants and data collection was outlined in Chapter 3.

4.3.1 Outcome measure

The outcome measure is the diagnosis of acute Infectious Mononucleosis caused by EBV. The gold standard reference test for this was positive EBV serology, more specifically positive anti-VCA (viral capsid antigen) IgM (Immunoglobulin M). See also Section 3.2.5.

4.3.2 Explanatory variables

Potential explanatory variables considered for inclusion in the derivation model were based on the four most likely clinical variables (presence of enlarged posterior cervical lymph nodes, presence of enlarged inguinal or axillary lymph nodes, palatine petechiae, splenomegaly) from a systematic review of signs and symptoms for the diagnosis of infectious mononucleosis, along with a further two variables; tonsillar exudate and objective fever, based on consensus between clinical colleagues.(177) Thus, based on the literature, and clinical consensus, the six variables judged to be most likely to be predictive were presence of enlarged posterior cervical lymph nodes, presence of enlarged inguinal or axillary lymph nodes, palatine petechiae, splenomegaly, exudate on tonsils, and temperature >38 degrees Celsius. As recommended for validation studies, data was also collected on several additional clinical variables including clinician's impression of diagnosis based on clinical likelihood of association with IM and to reduce the chance of omitting a variable that may have proved predictive. The full list of variables is listed in Table 4-1.

A variable that was found to be highly predictive according to the literature in this area was 'atypical lymphocytosis'.(40) However, this was not included in the derivation model regression analysis as the result is not readily available during the patient's consultation (in Irish general practice and many other health systems) and is thus not practical to be included in a consultation-based CPR. Nonetheless, given its predictive capacity and value in test sequence, an additional analysis was performed, to facilitate clinicians' inclusion of this variable where available as a POCT or if decision can be deferred until results are available.(177)

4.3.3 Data collection:

As described in Section 3.2.3, data were collected during recruitment using a patient questionnaire (Appendix 9: Participant Questionnaire) to obtain information about symptoms, and a case report form (Appendix 10: Case Report Form (CRF)) containing information about clinical signs, which was completed by the recruiting physician. The full list of variables is listed in Table 4-1.

4.3.4 Statistical Analysis

There were three continuous variables: age, pulse, and respiratory rate. The respiratory rate variable was subdivided into three categories, and age and pulse were dichotomized to simplify score generation and enable ease of application in clinical practice.

4.3.4.1 Descriptive statistics

Initially, appropriate descriptive statistics (mean, standard deviation, frequency, percentage) were used to compare presence of variables in participants with and without the outcome of interest. Missing values for each factor were tabulated and multiple imputation considered. However, due to the relatively small number of missing values the analysis included only participants with complete data.

4.3.4.2 Univariable associations

An assessment of the prognostic effect of each variable was considered separately, using univariate logistic regression, with the results expressed as odds ratios (OR) and associated 95% confidence intervals (CIs). When a variable was found to be protective, the covariate evaluated was absence of the variable, so that resultant odds ratios were >1.

A threshold p-value of ≤ 0.15 in the univariate analysis, or variables which were considered important following literature review and / or consensus, were included in a multivariable logistic regression model.

4.3.4.3 Multivariable associations

Four multivariable models were developed and transformed to point-based rules, with each odds ratio rounded to the nearest integer. This points system was established to make complex statistical models convenient to clinicians by simplifying the estimation of risk.

Regression coefficients are mutually adjusted for the other predictors, and thus represent the contribution of each predictor to the probability of the outcome. The estimated probability for an individual without any of the predictors depends only on the intercept. As suggested in the literature, relevant predictors were chosen based on a combination of clinical experience and research in the area.(181) While there is no consensus regarding how predictors should be selected while developing the final model, the two most common approaches are 'full model' and 'predictor selection' approaches. In the full model approach, as applied here, all previously identified candidate predictors are included, and no further analysis is performed. This avoids the risk of potentially excluding non-significant predictors identified in the literature although may result in non-significant terms in the model. Regression coefficients are mutually adjusted for the other predictors, and thus represent the contribution of each predictor to the probability of the outcome.

In Model 1, all explanatory variables of prior clinical importance or associated with a threshold p-value of ≤ 0.15 in the univariable analysis were included. In Model 2, the same variables were included as in Model 1, but a backward selection was used with a p-value threshold of 0.05. Stepwise backward regression was used to optimize the prediction model through simplification. This simplification improves the practicality of use for the physician, by reducing the number of values to input.

It was felt that the variable clinical impression could have been subjective between clinicians (especially those in a 'regular' general practice, who wouldn't encounter IM as frequently as recruiting clinicians in this study) and two further models (Models 3 and 4) were examined by excluding clinician impression from Model 1 and Model 2 respectively.

4.3.4.4 Discrimination and calibration of models

Ideally, an excellent predictive model (CPR) will correctly identify all patients that develop the outcome of interest, and not misclassify any patients.(182)

As described in Section 1.6.3, CPRs should be tested in the original (internal validation) and a new (external validation) population for reliability and accuracy. It was planned to derive and to internally validate the CPR in separate portions of the study population. However, because the target ample size required was not reached, although not ideal, internal validation was carried out in the same cohort as derivation of the CPR.

There are two main ways to assess how well a predictive model performs: discrimination and calibration. Discrimination is a model's ability to differentiate between individuals at higher risk and lower risk of having an outcome. Rates of discrimination depend on the characteristics of the study population, and whether it is heterogenous or homogenous. For binary outcomes (as in these studies), discrimination is generally measured using the receiver operating characteristic (ROC) curve or C statistic.(183) Choosing a score threshold for the model as to whether the patient is at higher risk or lower risk of the outcome affects the rates of true and false positives for the model. If a lower threshold is decided upon, then true positive (sensitivity) and false positive rates will both be increased. Discrimination of the CPRs was quantified using the area under the ROC curve statistic (AUC) and 95% confidence interval. An AUC of 0.5 represents chance, 0.7–0.9 represents moderate discrimination and 1.0 represents perfect discrimination.(184)

Calibration is the agreement between the predicted absolute risk and the observed risk for the outcome. Calibration may be good for some risk strata within a model but not for others. Calibration is measured by assessing the predicted and observed risk at different points. In this study, calibration was assessed visually and using the Hosmer-Lemeshow statistical test for goodness of fit. The Hosmer-Lemeshow test examines whether the difference between the predicted and observed outcome rate can be explained by chance.

4.3.4.5 Likelihood ratios and post-test probabilities

Furthermore, likelihood ratios (LR), and hence post-test probabilities, were calculated for all the models, to assess whether using the prediction rule changed the probability of a positive diagnosis of EBV. If a LR is greater than 1, this indicates that EBV is more likely. Pre-test probability is the probability of EBV diagnosis before the clinical prediction rule is used. The pre-test probability was estimated to be 8%, on the basis that this was the rate found in the age range 16-20 years in a large study from 1995 and the study population here was young adults (with a mean age of 20.6 and median age of 20).(6)

A subsequent analysis was performed to incorporate the variable 'atypical lymphocytosis >10%' where it is available to a clinician. A LR for atypical lymphocytosis was calculated from the univariable analysis. This LR was applied in series, following the CPR, adopting a Bayesian approach.(133) The previously calculated post-test probabilities for all four models now became pre-test probabilities, and the LR for atypical lymphocytosis was applied to these to arrive at new post-test probabilities.

All analyses were conducted using Stata version 16.(185)

4.4 Results

4.4.1 Descriptive statistics and univariable associations

There were 348 participants recruited in total: 42 with positive EBV serology (i.e., positive anti-VCA IgM result), 286 with negative EBV serology, 12 with equivocal serology results, 1 with a positive Monospot test, 5 with a negative Monospot test for IM, and 2 with missing data for EBV diagnosis. The participants with equivocal EBV serology and with Monospot tests were excluded from this analysis, leaving 328 participants whose data could be used. The participants ages ranged from 17-39 years old, with the mean being 20.6 years old. The mean age in the EBV positive group was 20.7 years old, and the mean in the control group was 20.6 years old. There were 225 females, 122 males and one gender neutral person recruited. The proportion of cases was approximately 13% overall, but varied between recruiting sites, from 7-29%. This may have been accounted for by recruiting physicians in

one of the sites only recruiting those in whom they had a clinical suspicion of IM, despite broad inclusion criteria.

Table 4-1 demonstrates the proportion of individuals positive for EBV for individual symptoms or signs, in addition to the unadjusted associations.

Participants who were EBV positive were more likely to have a temperature >38 degrees Celsius, exudate on their pharynx, exudate on their tonsils, enlarged tender posterior cervical lymph nodes, splenomegaly, and atypical lymphocytosis. Negative associations were found with shortness of breath (SOB) and dizziness.

Based on univariable regression analysis there was evidence of an association (p value ≤ 0.15) between EBV positivity and the following variables: presence of enlarged / tender posterior cervical lymph nodes, presence of exudate on tonsils, presence of splenomegaly, absence of shortness of breath, absence of feeling generally unwell, presence of exudate on pharynx, atypical lymphocytosis and clinician impression.

Table 4-1: Summary of variables and univariable associations between explanatory variables and EBV positivity

Symptom / sign	EBV positive n=42 N (%)[a]	Total n=328 N (%)[a]	Univariable Analysis OR (95% CI)	P-value
Temperature >38 [b]	2 (6.5)	11 (3.4)	1.85 (0.38 - 9.00)	0.44
Exudate on tonsils [b]	25 (61.0)	162 (49.4)	1.68 (0.86 - 3.27)	0.13
Splenomegaly [b]	2 (5.1)	3 (0.9)	14.81 (1.31 - 167.37)	0.03
Palatal petechiae [b]	11 (27.5)	92 (28.1)	0.95 (0.45 – 1.98)	0.88
Enlarged / tender posterior cervical lymph nodes [b]	22 (52.4)	76 (23.2)	4.71 (2.40 – 9.23)	<0.01
Enlarged / tender inguinal lymph nodes [b]	2 (5.1)	15 (4.6)	0.96 (0.21 - 4.42)	0.96

Symptom / sign	EBV positive n=42 N (%)[a]	Total n=328 N (%)[a]	Univariable Analysis OR (95% CI)	P-value
Enlarged / tender axillary lymph nodes [b]	2 (5.1)	11 (3.4)	1.51 (0.32 – 7.28)	0.61
Gender [c] (Reference: male) **Males** **Females**	 15 (35.7) 26 (61.9)	 119 (36.4) 208 (63.6)	 0.99 (0.50 – 1.95)	0.98
Cough	18 (43.9)	148 (45.1)	0.93 (0.48 – 1.79)	0.82
Nasal congestion	21 (51.2)	185 (56.4)	0.77 (0.40 – 1.48)	0.43
Shortness of breath (Absence of shortness of breath for univariable analysis)	7 (17.1)	90 (27.4)	2.02 (0.86 – 4.73)	0.12
Feeling generally unwell (absence of feeling generally unwell for univariable analysis)	33 (80.5)	285 (86.9)	1.91 (0.81 – 4.49)	0.14
History of fever	28 (68.3)	198 (60.4)	1.41 (0.70 – 2.83)	0.34
Headache	27 (65.9)	192 (58.5)	1.39 (0.70 – 2.77)	0.35
Muscle aches	21 (51.2)	163 (49.7)	1.04 (0.54 – 2.00)	0.92
Sweats / chills	28 (68.3)	217 (66.2)	1.07 (0.53 – 2.16)	0.85
Fatigue	36 (87.8)	266 (81.1)	1.63 (0.61 – 4.35)	0.33
Not sleeping well	28 (68.3)	228 (69.5)	0.89 (0.44 – 1.81)	0.76
Dizziness	10 (25.0)	103 (31.4)	0.69 (0.32 – 1.46)	0.33
Poor appetite	22 (53.7)	196 (59.8)	0.73 (0.38 – 1.41)	0.35
Exudate on pharynx	18 (43.9)	77 (23.5)	3.00 (1.52 – 5.92)	<0.01
Enlarged anterior cervical lymph nodes	37 (88.1)	278 (84.8)	1.35 (0.50 – 3.63)	0.55

Symptom / sign	EBV positive n=42 N (%)[a]	Total n=328 N (%)[a]	Univariable Analysis OR (95% CI)	P-value
Tender anterior cervical lymph nodes	33 (78.6)	241 (73.5)	1.36 (0.62 – 2.97)	0.44
Hepatomegaly	0	4 (1.2)	N/A – only 4 positive (controls)	
Respiratory rate 0-11	2 (7.7)	6 (2.6)	Reference	
Respiratory rate 12-16	21 (80.8)	165 (71.4)	0.29 (0.50 – 1.69)	0.17
Respiratory rate 17-28	3 (11.5)	60 (26.0)	0.11 (0.01 – 0.82)	0.03
Atypical Lymphocytosis >10%	26 (63.4)	29 (8.8)	162.36 (44.11-597.57)	<0.01
Clinician impression EBV positive	12 (28.6)	29 (8.8)	8.13 (3.43 – 19.28)	<0.01
	Mean (SD)	Mean (SD)		
Age	20.69 (3.94)	20.57 (2.78)	1.02 (0.91 – 1.13)	0.78
Pulse	82.82 (13.92)	81.13 (11.36)	1.01 (0.98 – 1.05)	0.45

OR=Odds Ratio; CI= Confidence Interval

[a] Calculations exclude those with missing data for each variable.

[b] Clinically important variables identified in the literature and from clinical consensus.

[c] The gender variable from the gender-neutral person was omitted from analysis, as any associations found in relation to just one participant would not lead to accurate conclusions. However, all other data from this participant was used.

4.4.2 Multivariable associations and developing CPR

Four variables of prior clinical importance (presence of a temperature >38 degrees Celsius, presence of palatal petechiae, absence of enlarged / tender inguinal lymph nodes, presence of enlarged / tender axillary lymph nodes) and seven variables with a threshold p-value of ≤ 0.15 in the univariable analysis (presence of enlarged / tender posterior cervical lymph nodes, presence of exudate on tonsils, presence of splenomegaly, absence of shortness of breath, absence of feeling generally unwell, presence of exudate on pharynx and clinician impression), were included in the first multivariable model (Model 1). The results (expressed as adjusted odds ratio, 95% confidence intervals and p values) are presented in Table 4-2.

As mentioned in Section 4.3.4, backward selection was also used to improve the practicality of use of the CPR for the physician, by reducing the number of values to input. Model 2 was a modification of Model 1 to include a backward selection ($p \leq 0.05$). This final model included two variables; presence of enlarged / tender posterior cervical lymph nodes and clinician impression.

Models 3 and 4 excluded the clinician impression variable. There was a total of 10 variables included in Model 3 (Model 1 excluding clinician impression).

Model 4 was a modification of Model 3 using a backward selection with $p \leq 0.05$. The final model included two variables; presence of enlarged / tender posterior cervical lymph nodes and presence of exudate on pharynx.

For all models, points were calculated for their explanatory variables, weighted appropriately.

Table 4-2: Multivariable analysis; models 1-4

	Model 1 a		Model 2 b		Model 3 c		Model 4 d	
Explanatory variable	**aOR (95%CI)**	**Points**	**aOR (95%CI)**	**Points**	**aOR (95%CI)**	**Points**	**aOR (95%CI)**	**Points**
Presence of a temperature >38	3.14 (0.51 – 19.43)	3			3.11 (0.54 - 17.82)	3		
Presence of exudate on tonsils	1.32 (0.44 – 3.98)	1			1.06 (0.38 – 2.97)	1		
Presence of splenomegaly	7.26 (0.21 – 252.43)	7			32.97 (0.83 – 1310.93)	33		
Presence of palatal petechiae	1.24 (0.42 – 3.66)	1			1.18 (0.45 – 3.11)	1		
Presence of enlarged / tender posterior cervical lymph nodes	5.63 (2.00 – 15.80)	6	5.30 (2.04 – 13.79)	5	4.97 (1.93 – 12.81)	5	4.77 (1.98 – 11.44)	5
Absence of enlarged / tender inguinal lymph nodes	1.49 (0.11 – 19.54)	1			1.66 (0.10 – 28.05)	2		
Presence of enlarged / tender axillary lymph nodes	1.43 (0.11 – 18.56)	1			1.64 (0.11 – 23.31)	2		
Absence of shortness of breath	3.97 (0.84 – 18.56)	4			3.24 (0.84 –	3		

	Model 1 a		Model 2 b		Model 3 c		Model 4 d	
Explanatory variable	**aOR (95%CI)**	**Points**	**aOR (95%CI)**	**Points**	**aOR (95%CI)**	**Points**	**aOR (95%CI)**	**Points**
					12.57)			
Feeling generally unwell	3.08 (0.79 – 11.91)	3			2.26 (0.63 – 8.16)	2		
Clinician impression	6.80 (1.60-29.00)	7	4.71 (1.52-14.59)	5				
Presence of exudate on pharynx	1.99 (0.60 – 6.64)	2			2.55 (0.85 – 7.63)	3	2.65 (1.08 – 6.52)	3
Hosmer-Lemeshow goodness-of-fit	$\chi 2$ =4.34 p=0.6307		$\chi 2$ =0.07 p=0.7962		$\chi 2$ = 0.504; p=0.54		$\chi 2$ = 0.7315; p=0.63	
Area under the receiver operating characteristics curve (95% confidence interval)	0.80 (0.69 – 0.91)		0.73 (0.63 – 0.82)		0.78 (0.68 – 0.87)		0.70 (0.62 – 0.79)	

OR=Odds Ratio; CI= Confidence Interval; aOR= Adjusted Odds Ratio

[a] Explanatory variables had to be of prior clinical importance or be associated with a threshold p-value of ≤ 0.15 in univariate analysis

[b] Explanatory variables had to be of prior clinical importance or be associated with a threshold p-value of ≤ 0.15 in univariate analysis, and then a backward selection was used with a p-value threshold of 0.05

[c] Explanatory variables had to be of prior clinical importance or be associated with a threshold p-value of ≤ 0.15 in univariate analysis. Clinician impression was not used in this model

[d] Explanatory variables had to be of prior clinical importance or be associated with a threshold p-value of ≤ 0.15 in univariate analysis, and then a backward selection was used with a p-value threshold of 0.05. Clinician impression was not included in this model

4.4.3 Discrimination of models

For each participant the EBV CPR score was calculated. The discrimination of all models is presented in Figure 4-1. The AUC values ranged from 0.70 - 0.80 (Model 1: 0.80 (95% CI 0.69 – 0.91), Model 2: 0.73 (95% CI 0.63 – 0.82), Model 3: 0.78 (95% CI 0.68 – 0.87), Model 4: 0.70 (95% CI 0.62 – 0.79)) all indicating moderate discrimination between those with and those without EBV.

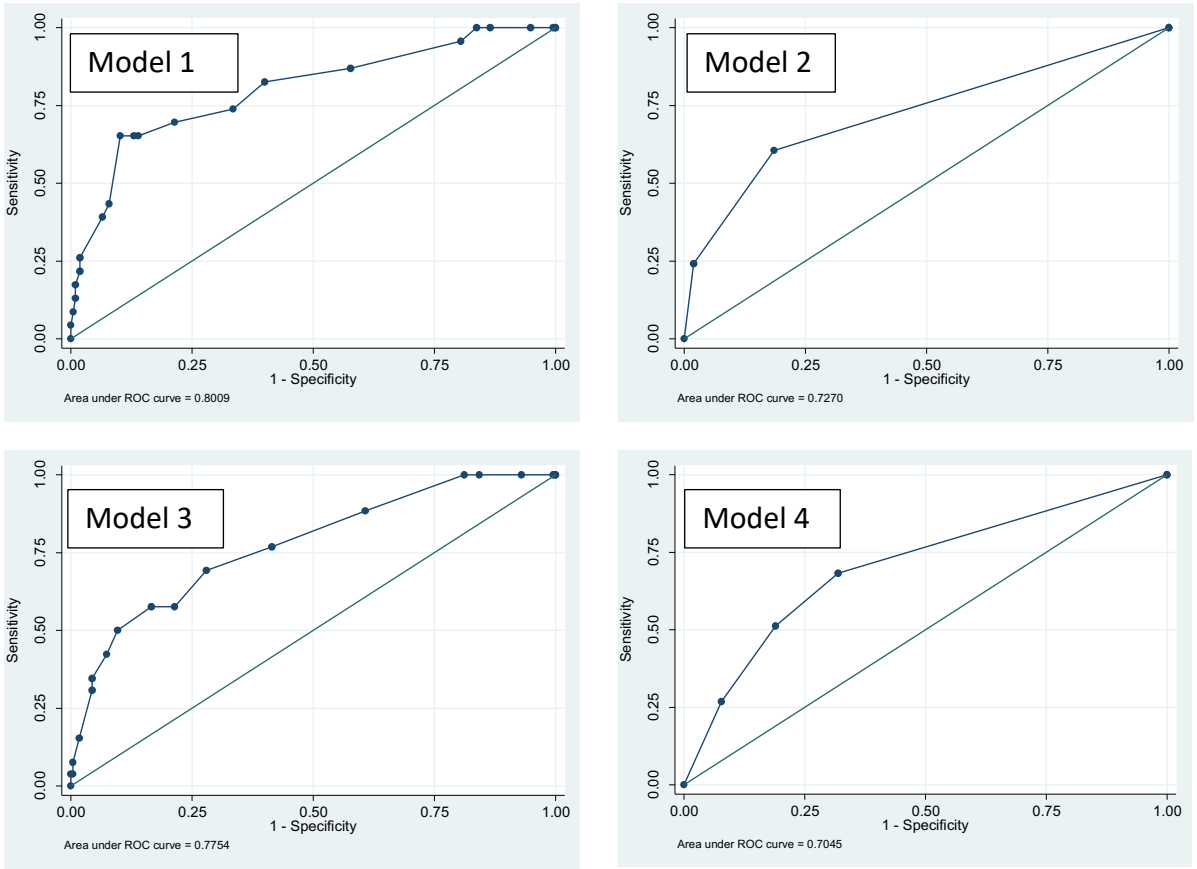

Figure 4-1: Receiver operating curve (ROC) for Models 1-4; a graph of the sensitivity (y-axis) and the specificity (x-axis).

4.4.4 Calibration of models

The calibration of all models is presented in Figure 4-2. The Hosmer-Lemeshow goodness-of-fit showed no evidence of a significant difference between the expected and the observed risk of EBV for all models, indicating good calibration.

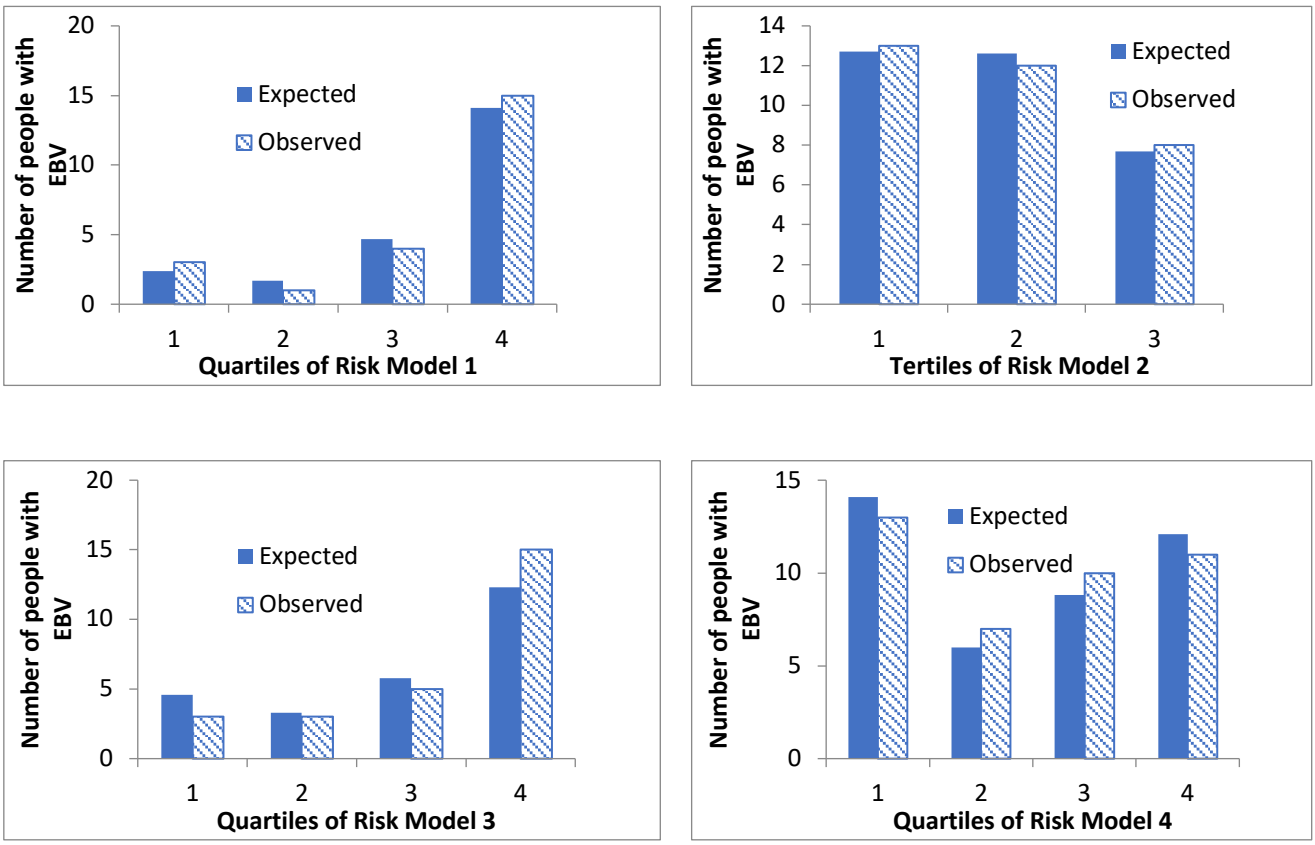

Figure 4-2: Observed and expected risk of EBV for Models 1-4

4.4.5 Likelihood ratios and post-test probabilities

Table 4-3 demonstrates the positive likelihood ratios and post-test probabilities for Models 1 and 3 (Model 1 had eleven variables with varying points scores, and Model 3 is the same as Model 1 but without the clinician impression variable.) The pre-test probability was estimated at 8% based on the literature, and the young adult study population (see also Section 4.3.4.5). There are wide confidence intervals for most of the post-test probabilities, which is unsurprising given the small number of positive cases.

Table 4-3: Likelihood ratios and post-test probabilities associated with the total scores for positive EBV (Model 1 and Model 3)

Model 1			Model 3		
Score	LR+	Post-test probability % * (95% CI)	**Score**	LR+	Post-test probability % * (95% CI)
≥0	1.00	8.0 (4.6-11.5)	**≥0**	1.00	8.0 (4.7-11.3)
≥1	1.00	8.0 (4.5-11.5)	**≥2**	1.00	8.0 (4.7-11.4)
≥2	1.05	8.4 (4.6-12.1)	**≥3**	1.08	8.5 (5.0-12.1)
≥3	1.16	9.2 (5.2-13.1)	**≥4**	1.19	9.4 (5.5-13.2)
≥4	1.19	9.4 (5.3-13.5)	**≥5**	1.23	9.7 (5.7-13.7)
≥5	1.19	9.4 (4.6-14.2)	**≥6**	1.46	11.3 (6.4-16.1)
≥6	1.51	11.6 (5.4-17.8)	**≥7**	1.85	13.9 (7.5-20.3)
≥7	2.07	15.3 (7.7-22.8)	**≥8**	2.48	17.7 (9.3-26.2)
≥8	2.21	16.1 (6.8-25.5)	**≥9**	2.70	19.0 (9.2-28.8)
≥9	3.25	22.0 (9.7-34.4)	**≥10**	3.48	23.2 (11.6-34.8)
≥10	4.67	28.9 (15.1-42.7)	**≥11**	5.20	31.2 (15.5-46.8)
≥11	5.01	30.4 (15.2-45.5)	**≥12**	5.70	33.1 (15.4-50.9)
≥12	6.37	35.7 (17.2-54.1)	**≥13**	7.93	40.8 (9.2-28.8)
≥13	5.50	32.4 (12.8-51.9)	**≥14**	7.05	38.0 (15.1-60.9)
≥14	6.01	34.3 (4.3-64.4)	**≥15**	8.81	43.4 (8.3-78.4)
≥15	14.02	54.9 (21.8-88.1)	**≥37**	17.62	60.5 (4.1-99.9)
≥16	11.68	50.4 (9.6-91.2)	**≥39**	8.81	43.4 (0.01-99.9)
≥17	18.70	61.9 (18.5-99.9)			
≥18	14.02	54.9 (0.0-99.9)			
≥21	18.70	61.9 (0.0-99.9)			

* Based on pre-test probability of 8%

Table 4-4 demonstrates the positive likelihood ratios (LR+) and post-test probabilities associated with Model 2 (presence of enlarged / tender posterior cervical lymph nodes and clinician impression) and Model 4 (presence of enlarged / tender posterior cervical lymph nodes and presence of exudate on pharynx). Again, the confidence intervals are very wide, especially for the higher scores.

Table 4-4: Likelihood ratios and post-test probabilities associated with the total scores for positive EBV (Model 2 and Model 4)

Model 2			**Model 4**		
Score	LR+	Post-test probability % * (95% CI)	Score	LR+	Post-test probability % * (95% CI)
≥0	1	8.0 (4.8-11.2)	≥0	1	8.0 (5.08-10.92)
			≥3	2.1388	15.7 (9.15-22.21)
≥5	3.2702	22.1 (12.1-32.2)	≥5	2.7033	19.0 (10.03-28.04)
≥10	12.5576	52.2 (24.5-79.9)	≥8	3.4756	23.2 (8.51-37.91)

*Based on pre-test probability of 8%

As outlined in Section 4.3.4.5, a subsequent univariable analysis was performed to explore the variable 'atypical lymphocytosis >10%'. This resulted in a positive likelihood ratio of 60.03 which was applied in series, following the CPR, to all four models, see Table 4-5 and Table 4-6.

Table 4-5: Post-test probabilities incorporating atypical lymphocytosis for positive EBV (Model 1 and Model 3)

Model 1			Model 3		
Score	Pre-test probability %* (95% CI)	Post-test probability %^ (95% CI)	**Score**	Pre-test probability %* (95% CI)	Post-test probability %^ (95% CI)
≥0	8.0 (4.6-11.5)	83.9 (79.2-88.6)	**≥0**	8.0 (4.7-11.3)	83.9 (79.4-88.4)
≥1	8.0 (4.5-11.5)	83.9 (79.2-88.7)	**≥2**	8.0 (4.7-11.4)	84.0 (79.5-88.5)
≥2	8.4 (4.6-12.1)	84.6 (79.7-89.5)	**≥3**	8.5 (5.0-12.1)	84.9 (80.4-89.4
≥3	9.2 (5.2-13.1)	85.8 (81.0-90.6)	**≥4**	9.4 (5.5-13.2)	86.1 (81.5-90.7)
≥4	9.4 (5.3-13.5)	86.1 (81.3-91.0)	**≥5**	9.7 (5.7-13.7)	86.5 (81.9-91.1)
≥5	9.4 (4.6-14.2)	86.1 (80.4-91.8)	**≥6**	11.3 (6.4-16.1)	88.4 (83.4-93.3)
≥6	11.6 (5.4-17.8)	88.7 (82.6-94.9)	**≥7**	13.9 (7.5-20.3)	90.6 (85.3-96.0)
≥7	15.3 (7.7-22.8)	91.5 (85.7-97.4)	**≥8**	17.7 (9.3-26.2)	92.8 (87.1-98.5)
≥8	16.1 (6.8-25.5)	92.0 (85.1-98.9)	**≥9**	19.0 (9.2-28.8)	93.4 (87.1-99.6)
≥9	22.0 (9.7-34.4)	94.4 (87.6-100)	**≥10**	23.2 (11.6-34.8)	94.8 (88.7-100)
≥10	28.9 (15.1-42.7)	96.1 (90.1-100)	**≥11**	31.2 (15.5-46.8)	96.4 (90.2-100)
≥11	30.4 (15.2-45.5)	96.3 (90.1-100)	**≥12**	33.1 (15.4-50.9)	96.7 (90.0-100)
≥12	35.7 (17.2-54.1)	97.1 (90.6-100)	**≥13**	40.8 (9.2-28.8)	97.6 (90.7-100)
≥13	32.4 (12.8-51.9)	96.6 (89.1-100)	**≥14**	38.0 (15.1-60.9)	97.4 (89.8-100)
≥14	34.3 (4.3-64.4)	96.9 (86.0-100)	**≥15**	43.4 (8.3-78.4)	97.9 (87.7-100)
≥15	54.9 (21.8-88.1)	98.7 (91.0-100)	**≥37**	60.5 (4.1-99.9)	98.9 (87.0-100)
≥16	50.4 (9.6-91.2)	98.4 (88.1-100)	**≥39**	43.4 (0.01-99.9)	97.9(77.5-100)
≥17	61.9 (18.5-99.9)	99.0 (90.0-100)			
≥18	54.9 (0.0-99.9)	98.7 (85.3-100)			
≥21	61.9 (0.0-99.9)	99.0 (78.9-100)			

* Pre-test probability is based on the results of application of the CPR (as calculated in Table 4.3)
^ Post-test probability is calculated using a LR+ of 60.03 for atypical lymphocytosis

Table 4-6: Post-test probabilities incorporating atypical lymphocytosis for positive EBV (Model 2 and Model 4)

Model 2			Model 4		
Score	Pre-test probability % * (95% CI)	Post-test probability % ^ (95% CI)	**Score**	Pre-test probability % * (95% CI)	Post-test probability % ^ (95% CI)
≥0	8.0 (4.8-11.2)	83.9 (79.6-88.2)	**≥0**	8.0 (5.08-10.92)	83.9 (80.0-87.9)
			≥3	15.7 (9.15-22.21)	91.8 (86.8-96.7)
≥5	22.1 (12.1-32.2)	94.5 (88.9-100)	**≥5**	19.0 (10.03-28.04)	93.4 (87.7-99.1)
≥10	52.2 (24.5-79.9)	98.5 (91.7-100)	**≥8**	23.2 (8.51-37.91)	94.8 (87.0-100)

* Pre-test probability is based on the results of application of the CPR (as calculated in Table 4.4)
^ Post-test probability is calculated using a LR+ of 60.03 for atypical lymphocytosis

4.5 Discussion

4.5.1 Main Results

This study prospectively gathered data on 348 patients (including 42 cases of IM) with sore throat presenting to primary care. Four multivariable clinical prediction models were derived for use in practice as point-based rules.

The four models, although all similar, have relative strengths and weaknesses. The AUC values for the four models ranged from 0.70 - 0.80 indicating moderate discrimination between those with and those without EBV. Models 1 and 3 provided slightly higher discrimination, and although they have many more variables than Models 2 and 4, they could be easily incorporated to a computer based clinical decision support system (CDSS), built into practice software. On the other hand, Models 2 and 4 still provided moderate discrimination and were simple to use in practice; with just two variables each to elucidate clinically and input to the CPR.

Using their maximum possible scores (i.e. all explanatory variables present), the CPR Models 1-4 produced varying post-test probabilities of 61.9%, 43.4%, 52.2% and 23.2% respectively.

Incorporating the variable atypical lymphocytosis (applied in series after the CPR) produced maximum post-test probabilities of 99.0%, 97.9%, 98.5%, 94.8% respectively. Even applying the atypical lymphocytosis to a low or zero CPR score produced a post-test probability of >80% (with relatively narrow confidence intervals) for all four models, which demonstrates the highly predictive nature of this variable.

The variable 'age' did not give any significant associations with the outcome, perhaps because the age range in our study population was quite narrow. The participants ages ranged from 17-39 years old, with the mean being 20.6 years old. Mean age for the group that were EBV positive was 20.7. However, it is known that IM is more prevalent in people in late adolescence or early adulthood dropping to 1% or less in people presenting with sore throat over the age of 35.(6)

4.5.2 Context of other studies

Although there is currently no widely used CPR for IM, many of the variables in our final models have been identified as being predictive in the previous literature in this area. In a 2021 systematic review of the predictive value of clinical examination and laboratory findings for IM, presence of palatine petechiae was found to have a positive likelihood ratio of 1.32-11.10, presence of posterior cervical adenopathy was found to have a positive likelihood ratio of 3.16, presence of axillary / inguinal adenopathy was found to have a positive likelihood ratio of 3.05, presence of presence of exudate on the tonsils had a positive likelihood ratio of 1.39-4.13, and presence of exudate on the pharynx had a positive likelihood ratio of 0.72-4.35.(40) The variable atypical lymphocytosis was found to have a positive likelihood ratio of 50.3 for $\geq$ 40% atypical lymphocytosis, 28.1 for $\geq$ 20% atypical lymphocytosis, and 8.97 for $\geq$ 10% atypical lymphocytosis. However, these previous findings were based on small numbers of original studies (for each variable) included in that systematic review. Clinical impression has not been described previously as being predictive for the diagnosis of infectious mononucleosis; however, it has been found to be predictive

for other acute respiratory conditions, e.g., rhinosinusitis and community acquired pneumonia.(128)

4.5.3 Clinical value / utility of different models

The study aim was to develop a prediction rule which would be straightforward to use in a clinical setting. CPRs, along with performing well in terms of predicting patients with a particular outcome, should be simple for clinicians to use. If eliciting symptoms and signs and inputting variables into a CPR is not easy to complete during a clinical consultation, the CPR will generally not be used.(121) It may be the case that the addition of extra explanatory variables will only slightly improve the accuracy of the CPR, at the expense of ease of its use. The use of CDSSs can overcome the issue of inputting the data; however, the explanatory variables still need to be collected via the patient's history and clinical examination.

In terms of their clinical utility, Models 1-4 varied in their maximum post-test probability; scoring 61.9%, 43.4%, 52.2% and 23.2% respectively. Referring back to test and treat thresholds (Section 1.7.3), it seems likely that clinicians would refer their patients for testing at the lower levels of probabilities (i.e. the maximum score for Models 2 and 4, and intermediate scores for Models 1 and 3), but might be confident to make a positive diagnosis and manage IM empirically at the upper levels of probabilities (in terms of advice concerning avoiding contact sports, and symptomatic treatment).

Incorporating atypical lymphocytosis, even with a low or zero CPR score produced a post-test probability of >80% for all models, demonstrating the highly predictive nature of this variable.

4.5.4 Implications for practice

Although the CPR that was developed is imperfect for diagnosis, it may perform an important function in helping clinicians to distinguish between patients in whom they should manage without a diagnostic test or whether they should test the patient for EBV. From literature, a person presenting with sore throat in the age range 16-20 years (which is a similar age profile to the current study population) has an approximately 7.9% chance of EBV being the causative agent.(6) Use of the CPRs can help to rule in or rule out a diagnosis of EBV IM, especially if they can be used in conjunction with an FBC. It needs to be borne in mind that the results presented here are based on a pre-test probability of 8% (as the study population was a young student health population). However, for many cases presenting in practice, the pre-test probability, and thus post-test probabilities will be lower. In practical terms, if none of the explanatory variables are present, the pre and resultant post-test probabilities could be < 5%, whereby the clinician could be confident to rule out the illness without further testing. In hindsight, and if the study were to be conducted on a larger scale, it would have been useful to ask clinicians for their test / treat thresholds for IM at the time of recruitment, as this would guide a more practical way of applying the results of the CPR.

Where readily available (ideally as POCT), the FBC could be used as an intermediate 'triage test' (see also Section 1.6.2). Clinicians may be satisfied that if it is positive, there is a strong likelihood that the patient has IM, although the literature suggests that relying on this predictor alone will lead to approximately one in seven cases of EBV IM being missed.(39) If the FBC indicates that atypical lymphocytosis is present (and particularly where there is a strong clinical suspicion of IM based on the CPR), EBV IM could be ruled in without the use of specific serology testing. However, if the FBC indicates no atypical lymphocytosis in the presence of clinical suspicion, EBV serology should be undertaken.

Although IM has no specific treatment other than supportive management, it is worthwhile to either make a firm positive diagnosis or rule it out. EBV serology testing is relatively inexpensive and low risk. Making a definitive diagnosis can be reassuring for the patient and clinician that there is no sinister underlying pathology causing adenopathy, it can give prognostic information in terms of recovery time; patients can be advised to avoid contact sports for eight weeks, and clinicians can be alerted to the possibility of complications from IM.

4.5.5 Future research

It was planned to split the dataset into a derivation and validation cohort. However, this was not possible, due to an insufficient number of EBV positive participants. Hence, the entire data was used in the derivation of the CPR. The CPR should undergo further robust prospective validation before it is deemed suitable for use in a clinical setting.

Clinical 'gestalt' / impression is a developing area of research in clinical medicine. It has been shown to have prognostic value in clinical settings, and, in some cases, performed better or similarly than CPRs especially for certain conditions.(128) The variable 'clinical impression' was shown in this study to predict the diagnosis of EBV reasonably accurately, when used in a CPR model with one other variable (Model 2). This demonstrates that clinical gestalt demonstrates reasonable predictive accuracy for EBV IM and this may be an area that future IM research could include.

4.5.6 Strengths and limitations of study

This study was a prospective cohort study of IM caused by EBV, based in primary care. There have not been many prospective cohort studies of this nature done previously. Furthermore, the study recruited participants from seven geographically distinct sites in Ireland.

However, some limitations were present. First, the study only examined infectious mononucleosis (IM) caused by EBV. EBV is the causative pathogen for approximately 90% of cases of IM and so although the findings will be applicable to most cases of IM, it will not be relevant for all, as the causative agent may be different in other cases. It was necessary, however, to focus on EBV for the purposes of this study as testing for and diagnosis of all pathogens was not feasible.

Additionally, given that the CPR for EBV IM was derived in an Irish adult primary care population it may not be readily generalizable to other settings e.g., hospitalised patients or children.

Although the total target number of participants was reached, the number of cases in this study was below target and relatively small at 42. The small number of participants meant that the study was developed and internally validated the CPR in the same population, which is likely to have given better results for internal validation than if other methods were used.

The study may be at risk of selection bias because participants were recruited from student health centres where we would expect participants to be relatively young and healthy prior to their current illness. However, this setting has been studied most frequently in the literature to date as it is a group that has a higher incidence of IM, and it is representative of the population we are trying to study. Another aspect of selection bias was that some recruitment sites had a higher rate of participants who were EBV positive and appeared to recruit participants who had symptoms suggestive of IM, rather than just using the broad inclusion criteria for all participants. This may have impacted on the results, in terms of the prior probability of the diagnosis being higher in sites where recruitment was more selective for those with features suggestive of IM.

Higher emphasis was placed in the analysis of increasing the post-test probability, i.e. ruling in a diagnosis of EBV IM. Given the relatively low pre-test probability of EBV IM, especially in patients over the age of 35, more emphasis should have been placed on decreasing the post-test probability, i.e. ruling out the diagnosis.(6) The method in which the variable atypical lymphocytosis was examined in series following the CPR has methodological flaws; in that, the likelihood ratio for this variable was derived from a univariable analysis, and then applied in series following a multivariable regression analysis. Therefore, these results should be interpreted with caution.

4.6 Conclusion

In conclusion, we have developed four simple clinical prediction models to help clinicians identify patients with acute cases of EBV IM. The prediction models, although not perfect for diagnosis, add to the body of evidence in this area. They may be useful tools to complement clinician's judgement, particularly in patients at intermediate ranges of probability in whom a decision needs to be made regarding confirmatory testing.

As this study is a derivation study, it is not appropriate to endorse use of the prediction model in clinical practice until further validation is performed.(121)

5 Chapter 5: External validation of a clinical prediction rule to aid in the diagnosis of IM

5.1 Introduction

As discussed in Section 1.6.3 , there are three main stages in the development of a CPR; derivation, validation (internal and external) and impact analysis. However, literature has shown that of the published CPRs, only about half had undergone validation and fewer still have undergone impact analysis.(186) This can happen for a variety of reasons, including cost, time constraints, lack of access to data.(187)

External validation is needed for two primary reasons: firstly, associations between explanatory variables and outcomes may occur because of chance, and secondly, the explanatory variables for the CPR may be particular to the population setting and may not be widely generalisable to a new setting. Spectrum bias refers to the effect a change in patient case mix or prevalence of disease between derivation and study populations may have on the performance of a CPR or a test.(188) Because of spectrum bias, external validation usually results in a reduction in performance of the CPR compared to that found in the original validation study.(189) However, if the external validation produces satisfactory results in a new population, this provides the clinician with confidence that the CPR can be used in a broad variety of settings.

5.2 Aims and objectives

The aim of this study was to externally validate the EBV CPR developed in Chapter 4 in a geographically separate population of patients and physicians. Validation of the EBV CPR in a different population tests the generalisability of the CPR and good performance of the CPR would support its use, thus allow for more accurate diagnosis of IM caused by EBV.

5.3 Methodology

A detailed description of the cohort study design, setting, participants and data collection of the original data set was outlined in Chapter 3. An international colleague with similar

research interests (Prof. Mark Ebell) had completed a Fulbright Scholarship in RCSI in 2019. At that stage we discussed collaborating on research in the area of IM. Prof. Ebell and his colleague, Dr. Xinyan Cai, were working on similar research to develop a CPR for IM in a US cohort of third level students. Sharing of this data allowed us to validate the CPR in a geographically separate population. The validation population had similar characteristics to the derivation cohort, but many of the variables collected in the derivation cohort were not present in the validation cohort. Hence, Model 4 was validated for pragmatic reasons. This model is simple to use; consisting of only two variables: presence of enlarged / tender posterior cervical lymph nodes and presence of exudate on pharynx.

5.3.1 Validation cohort

The validation cohort was based in a large student health centre in the University of Georgia, USA. Participants were included if their treating physician had a clinical suspicion of IM and ordered a diagnostic test. The population consisted almost entirely of people aged 18 to 25 years, with approximately 60% being female.

Dr. Cai had obtained de-identified data from the student health centre at University of Georgia for all patients in which IM was clinically suspected between September 1st, 2015, and January 1st, 2019, based on the fact that a heterophile antibody test (Monogen test) for IM was ordered. The student health centre at the University of Georgia provides primary care, specialty health care, education and prevention-focused services to approximately 35,000 students enrolled at the university each year. The student health centre uses an electronic health record to record the patients' symptoms, signs, and results of any investigations for each clinical visit. The health centre staff, who were not study team members, were responsible for linking the clinical and laboratory data and for removing any identifier/personal information, including name, age in years, birthdate, address, contact information, and student ID number.

5.3.2 Outcome measure (see also Section 3.2.5)

The outcome measure was the diagnosis of acute Infectious Mononucleosis caused by EBV. The reference standard test in the derivation population was EBV serology, which differed from the external validation population, where a heterophile antibody test was used for diagnosis. Ideally, the diagnostic tests in the derivation and validation populations would be the same, however, this was not possible in this study.

5.3.3 Sample size

This is discussed in Section 3.2.6.2. For the validation study, participants were included if they had a test for IM. Therefore, it was estimated that a sample size of approximately 300 would be required to yield 100 events of EBV IM.

5.3.4 Explanatory variables and weighting

The model that was used for external validation included two variables: presence of enlarged / tender posterior cervical lymph nodes and presence of exudate on pharynx, which were weighted 5 and 3 points respectively.

5.3.5 Statistical Analysis

Initially, appropriate descriptive statistics (mean, frequency, percentage) were used to compare presence of variables in participants with and without the outcome of interest in both the derivation and validation populations. Analysis included only participants with complete data. Data were analysed using Stata software for statistical analysis.(185)

For each participant the EBV CPR score was calculated. A value of 5 was assigned to presence of enlarged / tender posterior cervical lymph nodes and 3 was assigned to presence of exudate on pharynx. If neither of these variables were present, the score was 0. The total risk score was calculated by summing the values. Only participants with complete outcome data were included in the validation analysis. Two aspects of validity of the results were assessed; calibration and discrimination (as described in Section 4.3.4.4).

Finally, likelihood ratios (LR), and post-test probabilities, were calculated to assess whether using the prediction rule changed the probability of positive IM EBV diagnosis. If a LR is greater than 1, this indicates that IM caused by EBV is more likely. As in Chapter 4, the pre-test probability was estimated using the prevalence of EBV in this population, which was 8%, based on a large study from 1995, of patients in the age range 16-20 years presenting with sore throat.(6) However, the validation study differed, in that participants were included if their clinician had suspicion of IM and ordered a diagnostic test, thus the pre-test probability should be higher; the rate of IM in the validation cohort was 16%. Hence, a range of pre-test probabilities (8%-16%) was used with the likelihood ratios to calculate the post-test probabilities.

5.3.6 Derivation versus validation data

Both the derivation and validation populations consisted of young adults presenting with sore throat in a primary care student health setting. However, the inclusion criteria in the derivation and validation cohorts differed; in the derivation cohort the inclusion criteria were broader and included anyone with sore throat and one other symptom from: malaise, fatigue, lymphadenopathy, fever, headache. In the validation cohort; participants were included if the treating physician suspected IM and ordered a diagnostic test, so they were more likely to be diagnosed with IM, rather than other causes of sore throat. Explanatory variables were similar but not exactly the same in both populations. Outcome of IM was assessed by two different reference standards in the two populations; by EBV serology in the derivation population, and by a heterophile antibody test in the validation population. Comparison of the reference standards is covered in more detail in Section 1.2.5 and Section 3.2.5.

5.4 Results

The validation study containing 2342 participants in total was almost entirely people aged between 18 to 25 years, with approximately 60% being female. 379 (16.2%) had positive

heterophile antibody tests. The derivation data set has 348 participants of which 12.8% had a positive result for EBV serology. These rates are both higher than the rates found in previous studies, likely reflect the student health setting of the cohorts, where IM is known to be more prevalent.

5.4.1 *Summary of variables / symptoms*

Table 5-1 demonstrates a summary of the individual symptom or sign and positivity for IM in relation to the overall proportion of participants with that variable, for the two cohorts. Some of the variables vary greatly in their presence or absence between the two cohorts (e.g., presence of exudates on tonsils, headache, muscle ache, sweats / chills, fatigue), and this may be partly due to differences in examination technique, or confirmation bias on the part of the recruiting clinician, or participant who self-reports their symptoms. Other variables e.g., presence of enlarged / tender posterior cervical lymph nodes had very similar rates in the two cohorts. Another explanation for the differences in symptoms and signs between derivation and validation cohorts may be due to the inclusion criteria; sore throat and at least one other of the following symptoms: malaise, fatigue, lymphadenopathy, fever, headache in the derivation cohort, whereas the validation cohort was a prospective cohort of those who had a test requested for IM (irrespective of their symptoms). It is clear from the recruitment patterns in the various recruiting sites for the derivation cohorts (see also Section 3.4.1), that some sites recruited those who the physicians had more of a clinical suspicion of IM, and this may have impacted the proportion who had a particular symptom or sign also.

Table 5-1: Summary of variables and IM positivity

Symptom / sign	Derivation (EBV) IM positive group n=42 N (%)[a]	Total derivation group n=328 N (%)[a]	Validation IM positive group n=379 N (%)[a]	Total validation group n=2342 N (%)[a]
Temperature >38 [b]	2 (6.5)	11 (3.4)	11 (2.9)	153 (6.5)
Exudate on tonsils [b]	25 (61.0)	162 (49.4)	131 (34.6)	451 (19.3)
Enlarged / tender posterior cervical lymph nodes [b]	22 (52.4)	76 (23.2)	190 (50.1)	509 (21.7)
Cough	18 (43.9)	148 (45.1)	125 (33.0)	1012 (43.2)
Nasal congestion	21 (51.2)	185 (56.4)	163 (43.0)	1084 (46.3)
Headache	27 (65.9)	192 (58.5)	140 (36.9)	995 (42.5)
Muscle aches	21 (51.2)	163 (49.7)	83 (21.9)	657 (28.1)
Sweats / chills	28 (68.3)	217 (66.2)	159 (42.0)	964 (41.2)
Fatigue	36 (87.8)	266 (81.1)	195 (51.5)	1114 (47.6)
Exudate on pharynx	18 (43.9)	77 (23.5)	126 (33.2)	437 (18.7)
Enlarged / tender anterior cervical lymph nodes	38 (90.5)	284 (86.6)	312 (82.3)	1830 (78.1)
Atypical Lymphocytosis >10%	26 (63.4)	29 (8.8)	280 (73.9)	334 (14.3)

OR=Odds Ratio; CI= Confidence Interval

[a] Calculations exclude those with missing data for each variable.

[b] Clinically important variables identified in the literature and from clinical consensus.

5.4.2 Calculation of EBV CPR score in relation to cases of IM

The EBV CPR score was calculated for each individual in the validation cohort. Table 5-2 demonstrates a breakdown of the calculated EBV CPR score and rates of EBV positivity. For comparison, the first column demonstrates the rates in the derivation population. It is clear from the table that a high proportion of actual IM cases had a EBV CPR score of 0. However, the majority of cases (61%) had at least one of the explanatory variables. Almost 72% of negative cases were correctly identified as having a score of 0, with only 4% of negative cases having a score of 8.

Table 5-2: Breakdown of calculated EBV CPR score in validation cohort by positive/negative IM cases (n=2342)

EBV CPR Score	(EBV) IM positive in derivation cohort (%)	IM positive in validation cohort N (%)	Total validation cohort N (%)
0	31.7	147 (38.8)	1557 (66.5)
3	17.1	42 (11.1)	276 (11.8)
5	24.4	106 (28.0)	348 (14.9)
8	26.8	84 (22.2)	161 (6.9)
Total	100	379 (100)	2342(100)

5.4.3 Validation of EBV CPR for cases of IM

Figure 5-1 demonstrates that the AUC was 0.69 (95% CI: 0.67 to 0.72) indicating reasonable discrimination, and the Hosmer-Lemeshow goodness-of-fit showed no evidence of a significant difference between the expected and the observed risk of EBV ($\chi^2 = 3.40$; $p = 0.183$), indicating good calibration (Figure 5-2).

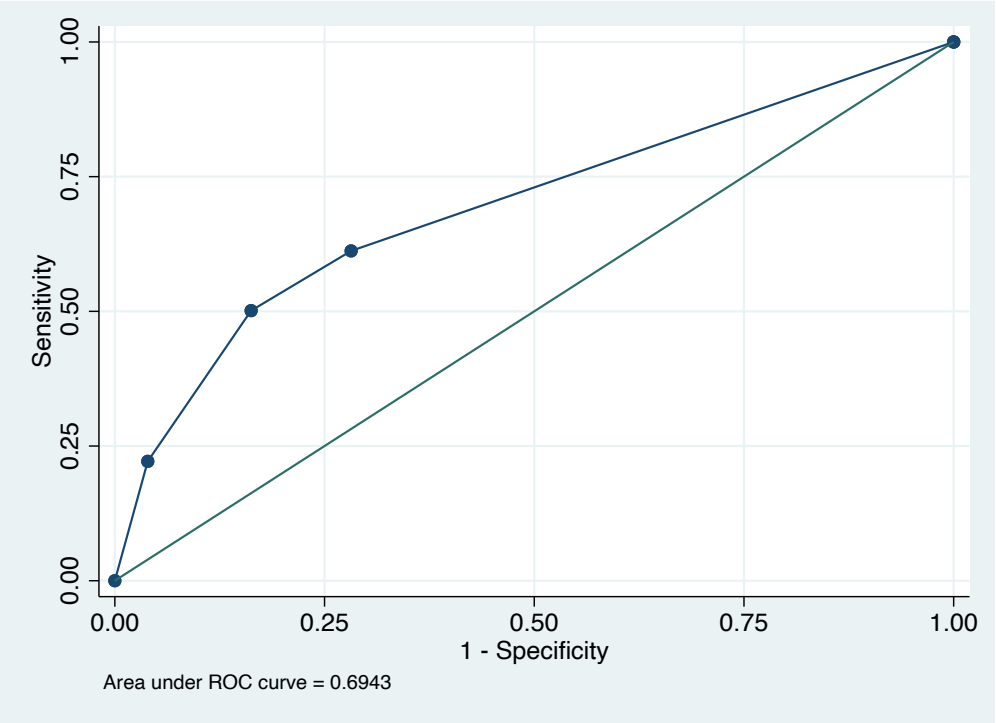

Figure 5-1: Receiver operating curve

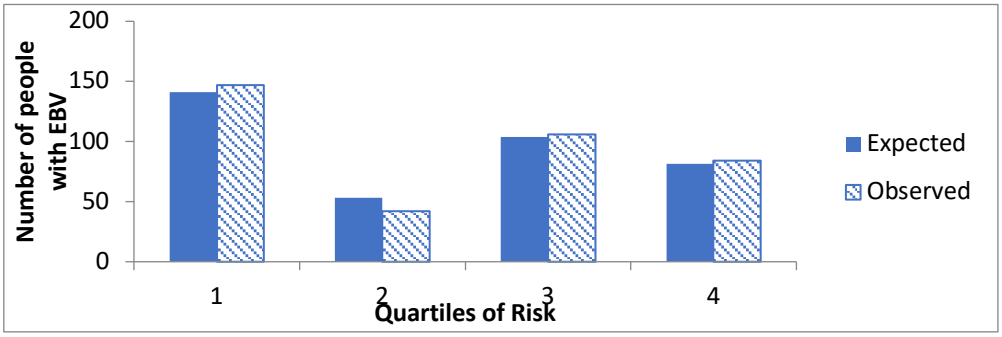

Figure 5-2: The expected and observed risk of IM by quartiles of predicted risk

To calculate post-test probabilities, a range of pre-test probabilities (prior) from 8-16% were used, as explained in Section 5.3.5. Table 5-3 demonstrates the post-test probabilities. The table demonstrates that if both explanatory variables are present, the probability of having acute IM ranges from approximately 33-52%, depending on which pre-test probability is chosen.

Table 5-3: Likelihood ratios and post-test probabilities for positive IM

Score	Positive Likelihood Ratio (LR+)	Post-test Prob (95% CI) 8% prior	Post-test Prob (95% CI) 12% prior	Post-test Prob (95% CI) 16% prior
≥0	1	8.0 (6.9-9.1)	12.0 (10.7-13.3)	16.0 (14.5-17.5)
≥3	2.1729	15.9 (13.3-18.5)	22.9 (19.9-25.9)	29.3 (26.0-32.5)
≥5	3.0849	21.2 (17.5-24.8)	29.6 (25.6-33.7)	38.0 (32.7-41.3)
≥8	5.6503	32.9 (25.5-40.4)	43.5 (35.7-51.3)	51.8 (44.0-59.7)

*LR+ is the likelihood ratio for a positive result, it is the ratio of the likelihood of a score of 0 or more among people with positive IM divided by the likelihood of a score of 0 or more among people with negative IM.

5.5 Discussion

5.5.1 Main Results

The simple, two item EBV CPR had reasonable discrimination and good calibration when validated in a separate population. The AUC was 0.69 (95% CI: 0.67 to 0.72) indicating reasonable discrimination, and the Hosmer-Lemeshow goodness-of-fit showed no evidence of a significant difference between the expected and the observed risk of EBV ($\chi^2 = 3.40$; $p = 0.183$), indicating good calibration. The post-test probabilities were calculated from a range of pre-test probabilities from 8%-16%, and the results suggest that the post-test probability could be up to approximately 50% if a patient has both relevant variables.

5.5.2 Context of other studies

Although there have been studies examining the predictive accuracy of signs and symptoms in cases of IM, there have been no previous CPRs developed and validated for use in patients with suspected IM.(40)

In terms of CPR validation studies; only approximately half of all CPRs have had any validation performed, and of these, validation is more likely to be internal than external.(186) Most CPRs do not predict outcomes as well in the validation population as

they do in their derivation population; however, our CPR performed similarly in the external validation population (AUC 0.69) as the internal validation (AUC 0.70).(189, 190)

5.5.3 Strengths and limitations of this research

This and the previous chapter describe deriving and externally validating a CPR for IM. This is novel, as there are no widely used CPRs for IM currently. An external validation was performed, in a large cohort, based in primary care. As previously mentioned, many existing CPRs have not been externally validated, and most do not perform as well in the validation population.

The study does have some limitations, however. As mentioned earlier, the derivation and validation cohorts differ in terms of their inclusion criteria, and also in terms of the diagnostic test used. The inclusion criteria of the validation cohort meant that a higher proportion of participants tested positive, however, lower sensitivity of the heterophile antibody test in the validation cohort may have resulted in more false negative results, so the true rate of EBV positivity in the validation cohort may have been higher.

When performing external validation of a CPR, it is recommended to perform it in a setting that is different from the derivation population.(186) This validation study was performed in a geographically distinct population in a different health system, but in a similar student health centre setting, which decreases the generalisability of the findings beyond the student/ young adult population.

5.5.4 Implications for practice

The CPR for IM has been derived and externally validated in a student health population and is suitable for use amongst a similar age range population in primary care. Using this validated two variable CPR for IM, if a young adult patient presents with sore throat and has the two predictive factors in the model, this increases their chance of EBV IM diagnosis to 33%-52% (with relatively wide confidence intervals), depending on their prior rate. If one or

both of the variables from the CPR is present, the patient may be categorised as intermediate risk of EBV IM diagnosis, and the clinician may be more inclined to perform a definitive test, whereas if neither variable is present, the patient may be categorised as low risk, allowing the clinician to adopt a 'watch and wait' approach.

5.5.5 Future research

While an external validation of the CPR was performed in a geographically distinct population, it was a population of university students, which was similar to the derivation study. The CPR would benefit from further large validation studies in more diverse populations, to assess whether it is suitable for use as a decision aid in patients presenting with sore throat.

5.6 Conclusion

In conclusion, this chapter described the external validation of a simple two item clinical prediction model derived in Chapter 4 to help clinicians identify patients with acute cases of EBV IM. It may be useful to guide clinicians in terms of which patients would benefit from further testing, and which patients are at low risk of having IM and could have an expectant management approach adopted.

6 Chapter 6: Centor Score clinical prediction rule validation

6.1 Introduction

6.1.1 Epidemiology

Sore throat / acute sore throat is one of the most commonly encountered presentations in primary care. Most cases are caused by viruses, and are self-limiting, however some cases are bacterial, and in severe cases, may be amenable to treatment with antibiotics. Group A Beta Haemolytic Streptococcus (GABHS) is the most common bacterial cause of acute sore throat in developed countries. A systematic review from 2000 examining the signs and symptoms that were most predictive of sore throat caused by GABHS, found rates of between 10-36% of GABHS in adults and children presenting with sore throat.(9) In the two large studies in the systematic review that included only adults, the rates were 10-15%.(3, 7) A similar rate (10.7%) was found in adults in another primary care cohort study.(191) A UK study from 2014, which used two cohorts (both including adults and children) in a clinical diagnostic study, found rates of GABHS of 31% and 23% in their cohorts.(180) Other bacterial causes include Group C / G Streptococcus and Fusobacterium necrophorum, which is also a known colonizer of the oropharynx.(192)

6.1.2 Clinical Features

A proportion of cases of acute sore throat have distinctive associated symptoms and signs depending on the cause, however, many are indistinguishable clinically. Sore throat caused by viruses is often associated with other features of an upper respiratory tract infection (URTI) e.g. cough, coryza and fatigue.(8) Generally, fever tends to be more prominent in bacterial rather than viral causes of sore throat.(78) A systematic review from 2000 examining the signs and symptoms that were most predictive of. GABHS sore throat, concluded that the most predictive variables were: presence of tonsillar exudate, pharyngeal exudate, exposure to GABHS infection in the previous 2 weeks, and the absence of tender anterior cervical nodes, tonsillar enlargement, or exudate.(9)

While usually a self-limiting condition, with symptoms lasting approximately 8-10 days, rare complications of GABHS include rheumatic fever, glomerulonephritis, otitis media, peritonsillar abscess and sinusitis.(9) The main goals of treating GABHS sore throat with antibiotics are to prevent complications, reduce spread of infection to others and to reduce duration and severity of symptoms.

Group C / G Streptococcus and Fusobacterium necrophorum are less frequent causes of sore throat but have similar symptoms to those of GABHS and can also rarely cause serious complications.

6.1.3 Diagnosis

The reference standard for diagnosis of GABHS is by throat swab culture.(77) However, the most recent European guidelines for the management of acute sore throat do not recommend throat swab as being necessary for the diagnosis of GABHS sore throat.(93) This is discussed in more detail in Section 1.3.3.

A variety of near patient tests / rapid antigen tests for GABHS have been developed. These give rapid results, and have high sensitivity and specificity, but they are not used routinely in primary care in Ireland.(193) A 2020 Cochrane review of the efficacy and safety of rapid tests to guide antibiotic prescriptions for sore throat, found that rapid testing reduces antibiotic prescription at an absolute rate of approximately 25% but may not have much impact on actual dispensing of antibiotics.(194) The aspect of antibiotic dispensing included only two studies, and the results were not statistically significant. The review concluded that more studies are required in this area.

6.1.4 Clinical prediction rules for GABHS sore throat

Many of the clinical guidelines for the diagnosis of GABHS incorporate clinical prediction rules (CPRs); mainly the Centor score, McIsaac score or the FeverPAIN score. Although the Centor score has been surpassed in terms newer CPRs detecting more people at low risk of

a diagnosis of GABHS, it is still widely used, possibly because if its simplicity. A 2019 survey 266 GPs in Denmark found that despite the McIsaac score being the recommended CPR in Denmark for diagnosis of GABHS, it was only used by approximately 15% of GPs, while the Centor score was used by approximately half of those surveyed.(195)

The Centor Score CPR was developed in 1981 to aid in the clinical diagnosis of GABHS sore throat, by stratifying patients into different risk categories.(74) The original score was derived in an emergency department setting, and based on the evaluation of 286 adults, over the age of 15. The score includes four variables: tonsillar exudates, swollen tender anterior cervical lymph nodes, absence of cough and history of fever. The aim of the CPR was to help clinicians distinguish between viral sore throat and sore throat caused by GABHS, and thus guide appropriate management, especially in terms of which patients may need to have a throat swab to confirm diagnosis, and whether antibiotics are indicated or not. Further research by the team that developed the original Centor Score CPR indicates that the score can be used for the diagnosis of groups C / G streptococcus and Fusobacterium necrophorum also.(196)

The Centor Score has undergone various modifications by different research teams. A similar CPR is the Modified / McIsaac Centor score.(197) This one is now more commonly used in practice. However, it uses slightly different variables to the Centor score and includes age as a variable, in order to account for the higher prevalence of GABHS in children. The five variables it uses are: age, exudate or swelling on tonsils, temperature >38 degrees Celsius, tender or swollen anterior cervical lymph nodes and absence of cough. It also specifies duration of symptoms as ≤ 3days, whereas the original Centor score didn't specify, although they did record duration of symptoms as one of their variables during the derivation process for the score.

Another variation which was developed in the UK in a larger derivation cohort and was deemed to perform better at identifying people at low risk of diagnosis of streptococcus is the FeverPAIN score.(180) The FeverPAIN score uses the five variables: fever in past 24 hours, absence of cough or coryza, symptom onset ≤3 days, purulent tonsils and severe tonsil inflammation. The FeverPAIN score can be used for diagnosis of group A, C or G

GABHS infection. The FeverPAIN score was assessed using an RCT which examined the outcomes of symptom severity ,duration of symptoms and use of antibiotics, and its use was found to improve symptoms reported and reduce antibiotic use.(90)

One further CPR (simplified Walsh score) uses similar variables, but includes exposure to GABHS sore throat as a variable.(7)

6.1.4.1 Performance of CPRs for GABHS sore throat

A 2011 systematic review examined the diagnostic accuracy of symptoms and signs and validation of the Centor score.(198) The review included 21 studies involving 4839 patients. The authors found that no individual symptom or sign had enough predictive power to rule in or rule out a diagnosis of GABHS sore throat, and that certain variables (fever and 'any exudates') were more valid for ruling in a diagnosis, while others (absence of cough and tender anterior cervical adenopathy) were more valid for ruling out a diagnosis. They indicated that, based on the findings of the systematic review, variables in the Centor Score could be given different weightings, depending on whether the clinician's aim was to rule in or rule out a diagnosis. It was concluded that the Centor score CPR was well calibrated, indicating that the rule is suitable for use in different settings, but, because of the low prevalence of GABHS in a primary care setting, it should be used with caution. A 2020 meta-analysis of ten primary care studies found that the Centor score only demonstrated fair discrimination (AUC of 0.69) and provided poor calibration for the diagnosis of GABHS sore throat.(199) The authors commented that the reporting of many of the included studies was inadequate and as a result it was difficult to assess their risk of bias. The study concluded that a score of ≤ 0 may be sufficient to rule out GABHS, but that point of care tests in addition to the CPR might be required to rule it in. The authors also concluded that guidelines for the diagnosis of GABHS, based on the use of the Centor and McIsaac scores, may be recommending the inappropriate prescription of antibiotics in a large number of cases.

A large-scale external validation of the McIsaac score in a retail health data setting in the USA achieved an AUC of 0.71, indicating good discrimination for patients aged 3 years and older.(200) Recently, a 2020 meta-analysis of eight primary care studies (reported in the same article as the meta-analysis of the Centor score above) demonstrated a similar result for AUC (0.705), but poor calibration, and similar to their conclusions for the Centor score, the authors stated that a score of ≤ 0 may be sufficient to rule out GABHS, but that point of care tests in conjunction with the CPR might be required to rule it in.(199) When a sensitivity analysis was carried out, excluding two studies that were led by the researcher who developed the McIsaac score, the AUC dropped to 0.67.

A very recent study examining the two CPRs that are used in the NICE guidelines (Centor and FeverPAIN) found that both CPRs performed poorly in a UK primary care population; resulting in an AUC of 0.62 for Centor, and 0.59 for FeverPAIN.(201) There were some limitations to the study, in that it was a secondary analysis of data, and not powered to validate CPRs. Also, due to the inclusion criteria, the spectrum of illness severity was narrow, and the authors commented that the CPRs were likely to have performed better in a broader spectrum population. There are no other external validations of the FeverPAIN score, to our knowledge.

External validation of the Walsh GABHS CPR in an urban population found an AUC of 0.71.(202)

The CPRs for the diagnosis of GABHS are summarised in Table 6-1.

Table 6-1: Comparison of GABHS CPRs

CPR name and variables used	Derivation Cohort	External Validation
Centor score(74) 1. tonsillar exudates 2. swollen tender anterior cervical lymph nodes 3. absence of cough 4. history of fever	286 participants > 15 years old USA	1. Systematic review concluded that Centor score was well calibrated, suitable for use in different settings, but should be used with caution in primary care setting(198) 2. Recent meta-analysis found that the Centor score only demonstrated fair discrimination (AUC of 0.69) and provided poor calibration for the diagnosis of GABHS sore throat.(199)
Modified / McIsaac Centor score(197) 1. age 2. exudate or swelling on tonsils 3. temperature >38 deg C 4. tender / swollen anterior cervical lymph nodes 5. absence of cough	521 participants ≥ 3 years old Canada	1. Large scale external validation in retail health data setting, indicated good discrimination.(200) 2. Recent meta-analysis demonstrated a similar result for AUC (0.705).(199) When a sensitivity analysis was carried out, excluding two studies by the researcher who developed McIsaac score, the AUC dropped to 0.67.
FeverPAIN score(180) 1. fever in past 24 hours 2. absence of cough or coryza 3. symptom onset ≤3 days 4. purulent tonsils 5. severe tonsil inflammation	1107 participants ≥ 3 years old UK	AUC 0.59 for FeverPAIN in UK primary care population(secondary data analysis).(201) Authors commented that the CPR likely to have performed better in broader spectrum population.
Simplified Walsh CPR(7) 1. cough 2. exposure to known streptococcal contact 3. temperature 4. tonsillar-pharyngeal exudates 5. cervical lymphadenopathy	171 participants ≥ 18 years old USA	External validation in an urban population found an AUC of 0.71(202)

6.2 Aims

6.2.1 Validation of Centor Score CPR for cases of GABHS sore throat

The main objective was to externally validate a CPR for GABHS sore throat. Ideally, both the FeverPAIN score and the Centor score would have been validated, however, the variable 'symptom onset ≤3 days' was not collected, so we were unable to validate the FeverPAIN score. The aim of this study was to externally validate the Centor score CPR for GABHS sore

throat in an Irish primary care population. Validation of the Centor score CPR in an Irish population would support its use and thus allow for more accurate diagnosis of GABHS sore throat and targeting of antibiotics to patients who are most likely to have this condition. Although the Centor score has been externally validated previously in several settings, it hasn't been validated in an Irish setting before, and doing so would allow investigation of whether the Centor score performs well in the type of patient encountered in Irish primary care. The participants in this study were recruited from student health centres in different locations in Ireland, and, in general, are young adults with few underlying health issues.

Building on the initial analysis, the variables in the Centor score were explored in our study population to investigate whether they should be given different weightings (updating the Centor score), or whether additional variables should be included (extending the Centor score) to improve its accuracy.

6.2.2 Validation of original Centor Score CPR in expanded study population of those with positive swabs for GABHS, Group C / G Streptococcus and Fusobacterium

The second aim of this chapter (which was a post hoc decision, based on results of throat swabs demonstrating a relatively high proportion of Group C/ G Streptococcus and Fusobacterium) was to externally validate the Centor score CPR in an expanded study population including those with positive swabs for GABHS, Group C / G Streptococcus and Fusobacterium.

6.3 Methodology

6.3.1 General methodology in relation to cohort study

Cohort set-up, study design, setting, participants and data collection are described in Chapter 3.

6.3.2 Outcome measures:

The outcome measure for the main study is the diagnosis of GABHS sore throat, in the context of sore throat presentation. The gold standard reference test for this is a throat swab which is positive for GABHS when cultured in the lab.

The outcome measure for the second study aim is the diagnosis of sore throat caused by either GABHS, Group C / G Streptococcus, or Fusobacterium. The gold standard reference test for these is a throat swab which tests positive for the relevant causative organism when cultured in the lab.

6.3.2.1 Variables

The variables of interest were the four original 'Centor Score Criteria'; tonsillar exudates, tender anterior cervical lymphadenopathy, absence of cough and a history of fever. In terms of extending the Centor score, the following variables were explored: clinician impression, age, pulse, and feeling generally unwell. Full details of the variables collected are outlined in Section 3.2.3.

6.3.3 Sample size

This is discussed in Section 3.2.6.3. It was estimated that a sample size of approximately 667 would be required to yield 100 events of GABHS (although the prevalence of GABHS on throat swabs in the original Centor score derivation study was 26%).(74)

6.3.4 Data collected:

Data was collected during recruitment as per the methods described in Chapter 3.

6.3.4.1 Derivation versus validation data

The derivation population consisted of 286 participants, over the age of 15 presenting with sore throat in an emergency department setting. The validation population consisted of 342

participants (with 80 positive cases of GABHS) over the age of 17 presenting with sore throat in a primary care student health setting. Explanatory variables and outcome assessment was the same in both populations.

6.3.5 Statistical Analysis

6.3.5.1 Validation of Centor Score CPR for cases of GABHS sore throat

Initially, appropriate descriptive statistics (mean, standard deviation, frequency, percentage) were used to describe the sample and compare presence of variables in participants with and without GABHS sore throat.

For each participant the Centor Score was calculated. A value of 1 was assigned to presence of tonsillar exudates, and 0 otherwise. The other variables were treated similarly; a value of 1 was assigned for tender anterior cervical lymphadenopathy, absence of cough and a history of fever. The total risk score was calculated by summing the values. Participants with complete outcome data were included in the validation analysis. Missing values for each variable were tabulated and due to the relatively small number of missing values the analysis included only participants with complete data. Two aspects of validity of the results were assessed; calibration and discrimination (as described in Section 4.3.4.4).

Secondly, the variables used in the Centor score were explored to assess whether the predictors had an effect that was clearly different in the validation sample. The third or final step was to assess whether including additional variables (clinician impression, age, pulse, feeling generally unwell) in the original Centor Score CPR improved the accuracy of the CPR in our study population. Overall performance (discrimination and calibration) of the new models was assessed using the Hosmer-Lemeshow test and AUC (see Section 3.1.7)

Finally, likelihood ratios (LR), and post-test probabilities were calculated to assess whether using the prediction rule changed the probability of positive GABHS sore throat. If a LR is greater than 1, this indicates positive GABHS is more likely. Pre-test probability (the probability of GABHS sore throat before the clinical prediction rule is used) was estimated

using the prevalence of GABHS sore throat in this population. The pre-test probability used in this study was 15%, on the basis that this was the upper limit of the rates found in adult studies, rates are known to be higher in children and younger adults, and this study population was young adults (with a mean age of 20.6).(3, 7)

6.3.5.2 Validation of original Centor Score CPR in expanded study population of those with positive swabs for GABHS, Group C / G Streptococcus and Fusobacterium

Similar methods to those described in Section 3.3.5.1 were used to assess the performance of the Centor score for GABHS, Group C / G Streptococcus and Fusobacterium. In brief, descriptive statistics were used to describe participants with and without GABHS, Group C / G Streptococcus and Fusobacterium. The Centor score was calculated for each participant and calibration and discrimination assessed using the Hosmer-Lemeshow test and AUC. Updating/extending of this model was not explored.

All analyses were conducted using Stata version 16.(185)

6.4 Results

Unfortunately, the target sample size was not achieved. There were 348 participants recruited in total: 80 with positive throat swabs for GABHS, 37 with positive throat swabs for Group C / G Streptococcus, 2 with positive throat swabs for streptococcus milleri, 2 with positive throat swabs for streptococcus anginosus, 2 with positive throat swabs for streptococcus B, 1 with positive throat swab for fusobacterium necrophorum, and 6 with missing data for throat swab result. The participants ages ranged from 17-39 years old, with the mean being 20.6 years old. There were 225 females, 122 males and one gender neutral person recruited. The rate of positivity for GABHS in our study population was 80/342 or 23.4%. This is higher than the rates found in previous studies, and may reflect carriage of GABHS, which is known to be higher in younger populations, and may be present in conjunction with acute viral sore throat.

6.4.1 Validation of Centor Score CPR (Model 1) for cases of GABHS sore throat

6.4.1.1 Summary of variables / symptoms

Table 6-2 demonstrates a summary of the individual symptom or sign and positivity for GABHS throat swab in relation to the overall proportion of participants with that variable.

Table 6-2: Summary of symptoms/variables by GABHS positive

Symptom / Sign	GABHS positive n=80 N(%)	GABHS negative n=262 N(%)	Total n=342 [a] N(%)
Gender [b], n(%)			
Males	24 (30.0)	94 (36.0)	118 (34.6)
Females	56 (70.0)	167 (64.0)	223 (65.4)
History of (hx) fever, n(%)	45 (56.3)	163 (62.2)	208 (60.8)
Absence cough, n(%)	53 (66.3)	133 (50.8)	186 (54.4)
Tender enlarged cervical LNs, n(%)	73 (91.3)	224 (85.5)	297 (86.8)
Tonsillar exudates, n(%)	44 (55.0)	126 (48.1)	170 (49.7)
Feeling generally unwell	70 (87.5)	228 (87.0)	298 (87.1)
Clinician impression GABHS positive, n(%)	56 (70.0)	111 (42.4)	167 (48.8)
Mean Age (SD)	21.0 (3.7)	20.4 (2.5)	20.6 (2.8)
Mean Pulse (SD)	81.6 (11.2)	81.1 (11.4)	81.1 (11.4)

[a] 6 participants with missing data excluded
[b] One gender neutral person was omitted from analysis, as any associations found in relation to just one participant would not lead to accurate conclusions. However, all other data from this participant was used.

6.4.1.2 Calculation of Centor Score in relation to cases of GABHS

The Centor Score was calculated for each individual in the cohort.

Table 6-3 demonstrates a breakdown of the calculated Centor score and GABHS. It is clear from the table that a higher proportion of actual cases had a Centor score of 2 or 3 rather than 4. However, only 9 actual cases of GABHS had a low score of 0 or 1. Ten participants were missing data for at least one of the variables included in the calculation of the Centor score and hence a total Centor score was not calculated.

Table 6-3: Breakdown of calculated Centor score (Model 1) by positive/negative GABHS cases (n=332)*

Centor Score	Probability positive culture original Centor study (%)	GABHS positive N (%)	GABHS negative N (%)	Total N (%)
0	2.5	2 (2.5)	12 (4.7)	14 (4.2)
1	6.0-6.9	7 (8.9)	42 (16.6)	49 (14.8)
2	14.1-16.6	25 (31.7)	66 (26.1)	91 (27.4)
3	30.1-34.1	24 (30.4)	80 (31.6)	104 (31.3)
4	55.7	21 (26.6)	53 (21.0)	74 (22.3)
Total	100	79 (100)	253 (100)	332(100)

* These figures are not consistent with total numbers with swab positive or negative for GABHS because of small amount missing data for Centor variables, so Centor score could only be calculated for 332 participants in total

6.4.1.3 Validation of original Centor Score CPR (Model 1) for cases of GABHS sore throat

Figure 6-1 demonstrates that while the AUC was 0.55 (95% confidence interval: 0.48 to 0.62) indicating poor discrimination, the Hosmer-Lemeshow goodness-of-fit showed no evidence of a significant difference between the expected and the observed risk of GABHS ($\chi^2 = 2.60$; $p = 0.273$), indicating good calibration (Figure 6-2).

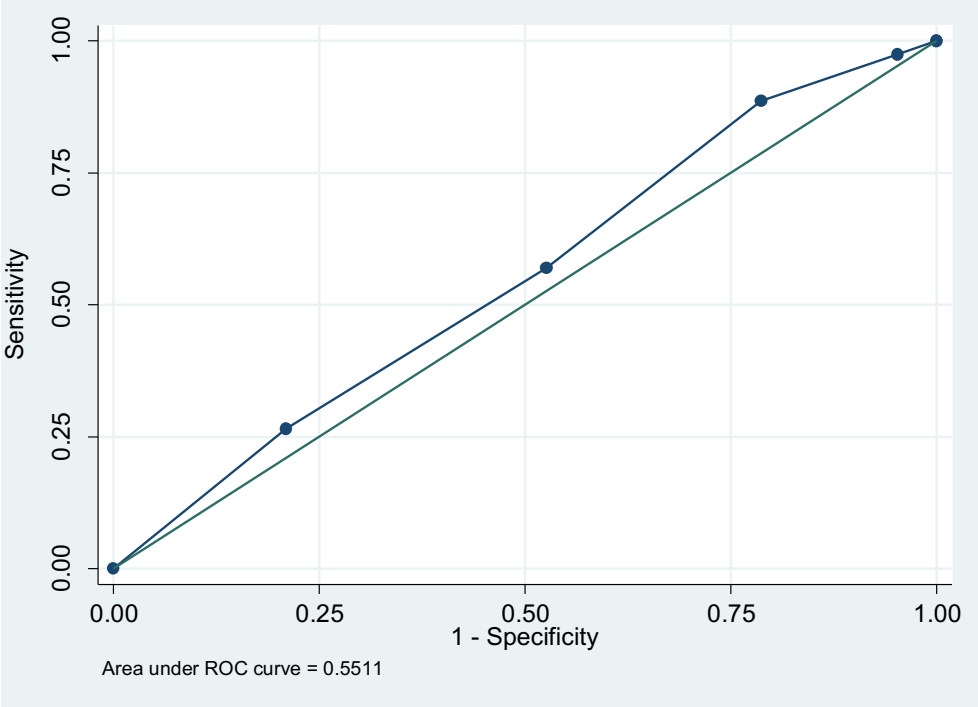

Figure 6-1: Receiver operating curve

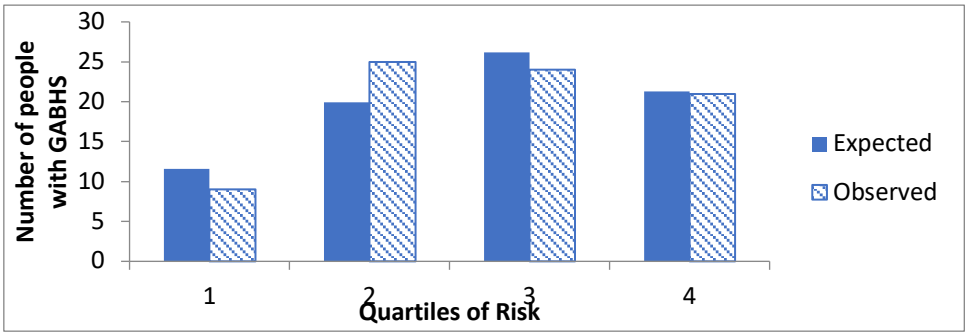

Figure 6-2: The expected and observed risk of GABHS by quartiles of predicted risk

To calculate post-test probabilities, we used 15% as the pre-test probability, as explained in Section 6.3.5.1 . Table 6-4 demonstrates the post-test probabilities. All LR values are between 1 and 1.3 indicating minimal increase in the likelihood of GABHS sore throat. For example, having a score of 4 or above using the clinical prediction rule, the probability of having GABHS sore throat increased just over 3% from 15.0% to 18.3%.

Table 6-4: Likelihood ratios and post-test probabilities for positive GABHS

Score	Positive Likelihood Ratio (LR+)	Post-test Probability (95% CI)
≥0	1.00*	15.0% (11.1 – 18.9)
≥1	1.02	15.3% (11.3 – 19.3)
≥2	1.13	16.6% (12.0 – 21.1)
≥3	1.08	16.1% (10.5 – 21.6)
≥4	1.27	18.3% (9.3 – 27.3)

*LR+ is the likelihood ratio for a positive result, it is the ratio of the likelihood of a score of 0 or more among people with positive GABHS divided by the likelihood of a score of 0 or more among people with negative GABHS. This first cut point (≥0) indicates that all patients with positive GABHS scored 0 or more. Consequently, all positive GABHS cases are correctly classified (sensitivity=100%), however, none of the negative GABHS patients are classified correctly (specificity=0%).

6.4.2 Update of Centor Score CPR in cases of GABHS

Variables in the Centor score were also tested to see if they had an effect that was clearly different in the validation sample. Likelihood ratio tests of the model updates showed evidence ($p<0.05$) that history of fever should be omitted, and absence of cough increased to 2 points (see Model 2, outlined in Table 6-5 below).

Table 6-5: Updated Centor Score CPR (Model 2) for study population

Variable	Points
Tonsillar exudates	1
Tender enlarged cervical LNs	1
Absence cough	2
Hx fever	0 (omitted)

The AUC, although it had a slight increase to 0.59 (95% confidence interval: 0.52 to 0.66), still indicated poor discrimination. Again, the Hosmer-Lemeshow goodness-of-fit showed no

evidence of a significant difference between the expected and the observed risk of GABHS ($\chi^2 = 0.13$; $p = 0.9365$) indicating very good calibration.

6.4.3 Extension of Centor Score CPR (Model 3) examining effects of additional variables in cases of GABHS sore throat

The third step was to assess whether including additional variables in the original Centor Score CPR improved the accuracy of the CPR in our study population. Clinician impression, age, pulse, feeling generally unwell were included in an extended model to examine whether the addition of any of these variables improved the model performance. Likelihood ratio tests were used to test the addition of each variable. There was no evidence of an improvement for age, pulse and feeling generally unwell. However, clinician impression was significantly associated with GABHS, as shown in Table 6-6.

Table 6-6: examining effects of additional variables to extend Centor Score CPR

Variable	χ test statistic, p-value
Age	χ=2.38, p=0.12
Pulse	χ=0.14, p=0.71
Feeling generally unwell	χ=0.00, p=0.99
Clinician impression	χ=12.22, p<0.01

6.4.3.1 Final model (Model 3) - associated risks

Having updated and extended the Centor Score, the final model (Model 3) included Tonsillar exudates (1 point (pt)), tender enlarged cervical LNs (1 pt), absence of a cough (2 pt) and clinical impression (1 pt).

Figure 6-3 demonstrates that discrimination, as measured by AUC, was still poor (AUC: 0.62; 95% CI: 0.55 to 0.69), but calibration was good (Hosmer-Lemeshow goodness-of-fit: χ^2 = 3.15; p = 0.3698)(Figure 6-4).

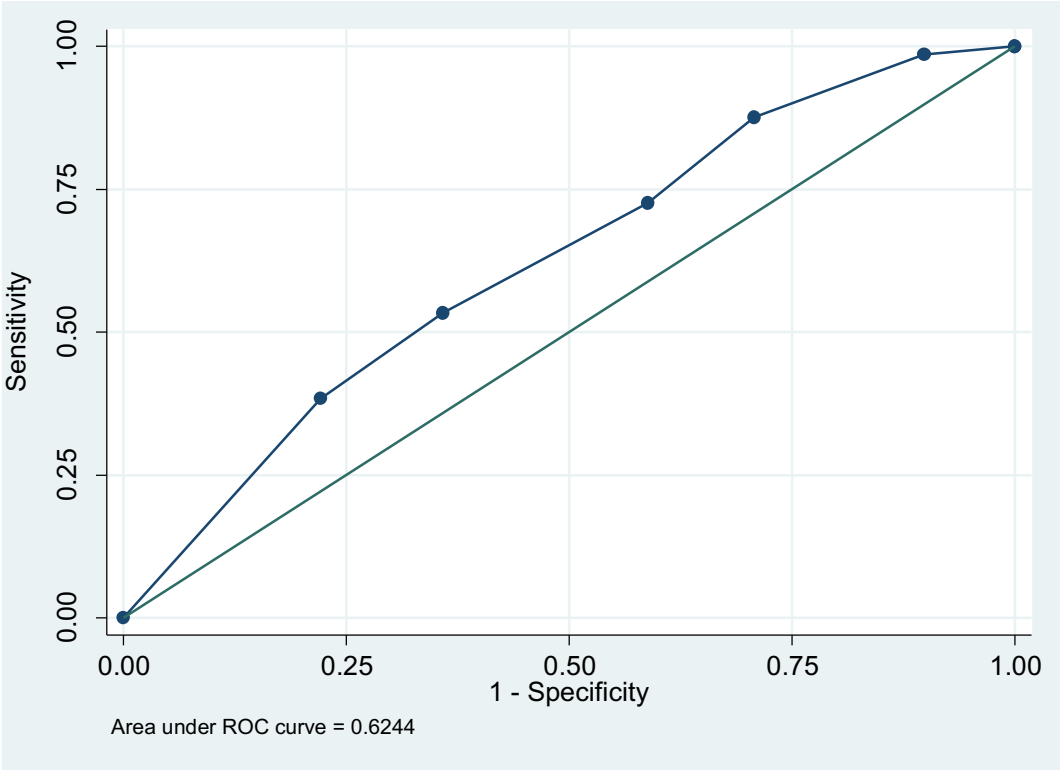

Figure 6-3: Receiver Operating Curve

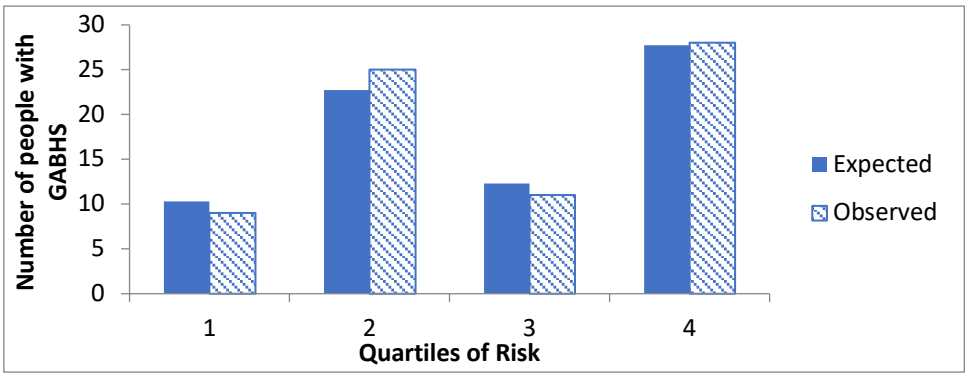

Figure 6-4: The expected and observed risk of GABHS by quartiles of predicted risk

To calculate post-test probabilities, we used 15% as the pre-test probability, as explained in Section 6.3.5.1 . Table 6-7 demonstrates the post-test probabilities. All LR values are

between 1 and 2 indicating minimal increase in the likelihood of GABHS sore throat. For example, having a score of 4 or above using the clinical prediction rule, the probability of having GABHS sore throat increased just over 5% from 15.0% to 20.8%.

Table 6-7: Likelihood ratios and post-test probabilities for positive GABHS

Score	Positive Likelihood Ratio (LR+)	Post-test Probability (95% CI)
≥0	1.00*	15.0% (10.3 – 19.8)
≥1	1.10	16.2% (11.1 – 21.4)
≥2	1.24	17.9% (11.9 – 24.0)
≥3	1.23	17.9% (11.2 – 24.5)
≥4	1.49	20.8% (11.8 – 29.9
≥5	1.73	23.4% (11.4 – 35.4)

*LR+ is the likelihood ratio for a positive result, it is the ratio of the likelihood of a score of 0 or more among people with positive GABHS divided by the likelihood of a score of 0 or more among people with negative GABHS. This first cut point (≥0) indicates that all patients with positive GABHS scored 0 or more. Consequently, all positive GABHS cases are correctly classified (sensitivity=100%), however, none of the negative GABHS patients are classified correctly (specificity=0%).

6.4.4 Validation of original Centor Score CPR in expanded study population

6.4.4.1 Summary of variables / symptoms from expanded study population (those with positive swabs for GABHS, Group C / G Streptococcus and Fusobacterium)

Table 6-8 demonstrates a summary of the individual variables, and positivity for GABHS, Group C / G Streptococcus, or Fusobacterium throat swab in relation to the overall proportion of participants with that variable.

Table 6-8: Summary of symptoms/variables by GABHS, Group C / G Streptococcus, fusobacterium positive

Symptom / Sign	GABHS, StreptococcusC/G, fuso positive n=118 N(%)	GABHS, StreptococcusC/G, fuso negative n=224 N(%)	Total n=342[a] N(%)
Gender[b], n(%)			
Males	38 (32.2)	80 (35.9)	118 (34.6)
Females	80 (67.8)	143 (64.1)	223 (65.4)
Hx fever, n(%)	75 (63.6)	133 (59.4)	208 (60.8)
Absence cough, n(%)	69 (58.5)	117 (52.2)	186 (54.4)
Tender enlarged cervical LNs, n(%)	106 (89.8)	191 (85.3)	297 (86.8)
Tonsillar exudates, n(%)	62 (52.5)	108 (48.2)	170 (49.7)
Clinician impression GABHS positive, n(%)	72 (61.0)	95 (42.4)	167 (48.8)
Mean Age (SD)	20.69 (3.30)	20.51 (2.49)	20.57 (2.78)

[a] 6 participants with missing data excluded
[b] One gender neutral person was omitted from analysis, as any associations found in relation to just one participant would not lead to accurate conclusions. However, all other data from this participant was used.

6.4.4.2 Validation of original Centor Score (Model 1) using positive swabs for GABHS, Group C / G Streptococcus and fusobacterium

Figure 6-5 demonstrates that the AUC was 0.54 (95% confidence interval: 0.48 to 0.60) indicating poor discrimination. However, the Hosmer-Lemeshow goodness-of-fit showed no evidence of a significant difference between the expected and the observed risk of GABHS, Group C / G Streptococcus and fusobacterium ($\chi^2 = 0.57$; $p = 0.451$), indicating good calibration (Figure 6-6).

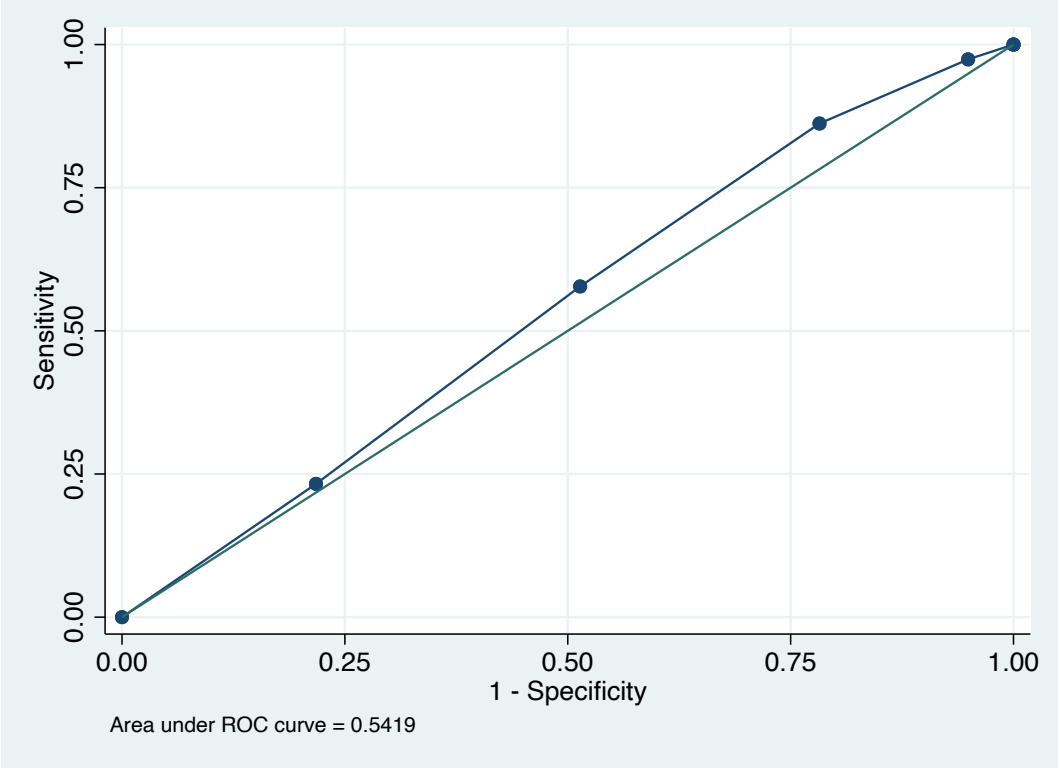

Figure 6-5: Receiver operating curve

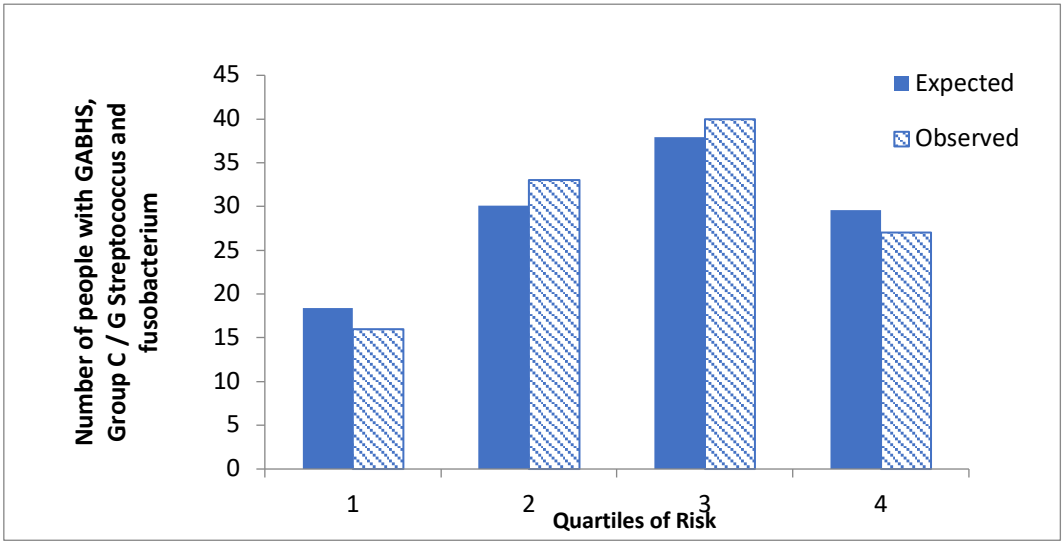

Figure 6-6: The expected and observed risk of GABHS, Group C / G Streptococcus and fusobacterium by quartiles of predicted risk

6.5 Discussion

6.5.1 Main results

The pre-test probability was estimated to be approximately 15%, in our young adult (mean age of 20.6) population presenting with sore throat, based on the fact that this was the upper limit of rates found in adult studies (5-15%), and rates are known to be higher in children and younger adults.(3, 7) However, the actual proportion of participants who had a positive throat swab was 23.4%, which very likely includes a significant number of GABHS carriers. The proportion of participants with positive swabs in the original (adolescent / young adult) derivation cohort was 17%.(74)

The original Centor score CPR (Model 1) had poor discrimination with an AUC was 0.55, but good calibration, when used in our validation population. The results suggest that although the CPR can act as a decision aid for testing and treating, the post-test probability is reasonably low, even with a high Centor score.

When the variables used in the original Centor score were tested to examine whether they had a different effect in our validation study population, the CPR was modified to omit history of fever, and to give a double weighting to the variable absence of cough (Model 2). This improved the calibration of the score, but discrimination remained poor.

Additional variables were then tested to examine whether their addition would improve the performance of the score; the only one that was found to make a difference was the variable clinician impression. This was added to Model 2 to give a final model (Model 3) with the variables: tonsillar exudates (1 pt), tender enlarged cervical lymph nodes (1 pt) absence of cough (2 pts), clinician impression (1 pt). This final model again had good calibration but poor discrimination.

The original Centor score CPR (Model 1) was also tested in an extended population, including not just participants with GABHS, but also those with Group C/G Streptococcus or Fusobacterium identified on their throat swab culture. The CPR resulted in good calibration but poor discrimination again.

6.5.2 Context of other studies

A 2011 systematic review of 21 studies examining the diagnostic accuracy of symptoms and signs and validation of the Centor score, found that although the Centor score CPR was well calibrated for use in different settings, because of the low prevalence of GABHS in a primary care setting, it should be used with caution.(198) A later large-scale validation of the Centor score was performed in a USA retail health chain population in 2012.(200) This study included >200,000 patients aged over 3 years old, who presented with sore throat to a retail health clinical setting. Although the validation population differed from the derivation population (retail health v's emergency department setting), the discrimination of the CPR was found to be good at 0.72 for patients aged 15 or older.

More recently, a 2020 meta-analysis of ten primary care studies found that the Centor score only demonstrated fair discrimination (AUC of 0.69) and resulted in poor calibration.(199) A subsequent study from 2021 validating the Centor score CPR similarly found that it performed poorly in a UK primary care population with an AUC of 0.62.(201) However, there were limitations to the study itself, in that it was not powered to validate CPRs, and used a validation population with a narrow spectrum of illness.

The result for discrimination of the Centor Score CPR was inferior in our validation population than in any of the above studies. There are a number of reasons why this may be the case. Our validation study population was relatively homogenous in terms of age profile (17-39 years old, mean being 20.57), small study population (80 positive cases), and high rate of GABHS positivity on throat swab (23.4%, rather than the expected 5-15%). The high rate of GABHS positivity can be at least partly accounted for by a high rate of GABHS carriage (which is known to be higher in younger adults). Also, examining the proportion of cases in our validation study population and the original Centor CPR derivation population that had Centor variables (
Table 6-3), the original derivation cases had a far greater proportion of Centor score 4, indicating more severe illness. Furthermore, they were recruited from an emergency department setting, which would indicate greater severity of illness than a primary care setting. High rates of GABHS carriage, along with spectrum bias resulting in differences between the original derivation study and the validation population might account for poor discrimination performance for the CPR in our validation study.(188)

6.5.3 Strengths and limitations of this research

This research was performed prospectively, in an Irish primary care setting, in which the Centor score has not been validated previously. As throat swab was used as the reference standard test, we were able to pick up cases of Group C/G Streptococcus and Fusobacterium Necrophorum and examine how the Centor score performed in that expanded population.

However, the study also has some limitations. It's based on a relatively small number of cases (80 cases of GABHS), which did not meet adequate sample size requisites (at least 100 cases and 100 non-cases are required) and the validation study population was relatively homogenous.

Additionally, it is probable that a significant number of participants included in this study were actually GABHS carriers rather than having acute GABHS, especially since the rate of GABHS found in the study was higher than that reported in the literature (on which the pre-test probability for the study was based). However, since all of the participants were symptomatic with sore throat (which also may have been due to viral causes), they were assumed to be true positives for GABHS, which likely overestimated the number of cases. It is difficult to know how many of the patients included in the derivation population cases were carriers rather than acute cases of GABHS.

6.5.4 Implications for practice

A large scale primary care study performed in the USA over a ten year period revealed that 73% of adults presenting with sore throat received a prescription for antibiotics.(203) This far surpasses the estimated prevalence of 5-15% for GABHS, and clearly antibiotics are being inappropriately prescribed for what is usually a mild self-limiting condition. An observational study from the UK from 2020 also demonstrated that while antibiotics were targeted more at higher risk patients, even patients with a low FeverPAIN CPR score for GABHS were being inappropriately prescribed antibiotics.(204)

This study adds to the existing literature in the area of external validation of CPRs for GABHS. Most recent external validations of the Centor and FeverPAIN CPRs have only demonstrated poor or fair diagnostic accuracy in the validation population, although they are widely used in international guidelines for sore throat. International clinical guidelines may need to be updated to reflect recent external validations of the Centor and FeverPAIN CPRs, and amended to recommend antibiotic prescribing only in severe high risk cases.

The improvement of clinical guidelines advocating the use of CPRs may be just one element of a broader approach.(205) A small study from 2017 demonstrated that reduction in the prescription of antibiotics was achieved through a multi-faceted approach involving the use of a multidisciplinary team, dissemination of guidelines and a change to GP record management for sore throat.(206)

6.5.5 Future research

Future research should focus on large scale primary care validation of the Centor and FeverPAIN score CPRs in a heterogenous population, with cases of varying severity.

6.6 Conclusion

The Centor Score CPR was developed 40 years ago and is still widely used to aid in the diagnosis of GABHS sore throat. Despite recent external validations (including the one described in this thesis) only demonstrating moderate or poor performance, it is used in a number of international clinical guidelines for sore throat and has been demonstrated to reduce antibiotic prescribing.(206)

This validation analysis showed good calibration but poor discrimination, most likely due to a high rate of GABHS carriage, as the reference standard used cannot discriminate between active illness and carriage.

7 Chapter 7: Observational study to explore potential associations between IM caused by EBV and fatigue, and time missed from college / work in younger adults.

7.1 Introduction

Most people who have acute IM recover spontaneously over 4-6 weeks but a small proportion report ongoing fatigue in the months following acute infection.(27, 207) Several studies have demonstrated rates of approximately 10% for prolonged fatigue after acute IM.(32, 33, 208, 209) However, many of these studies are outdated, were not performed prospectively, or did not use serological testing as their criterion for diagnosis of IM caused by EBV.

7.1.1 Chronic Fatigue Syndrome (CFS)

CFS was defined in 1994 as 'characterized by severe disabling fatigue and a combination of symptoms that prominently features self-reported impairments in concentration and short-term memory, sleep disturbances, and musculoskeletal pain'.(210) It is a diagnosis of exclusion, typically made after symptoms have been present for six months or more. Although the risk of CFS is much greater with EBV than other upper respiratory tract infections, it is not specific to EBV alone and has been shown to occur post parvovirus infection, Q fever and Ross River infections among others.(33, 211)

Later studies have concluded that CFS is more likely to be a group of heterogenous syndromes, or a spectrum of fatigue. A large population survey study of over 15,000 people in the UK concluded that fatigue is common and is continuously distributed in the community, with CFS at the upper end of the spectrum, rather than a discrete condition in itself.(212) The study also found that fatigue was present for more than six months in 18% of people and is closely associated with psychosocial factors. Women had a relative risk of fatigue being present of 1.3 relative to men in this study. Another study from 2006 suggests that CFS is a group of heterogenous syndromes that need further research in order to determine causes, pathophysiology and treatments.(213)

7.1.2 Studies examining IM and factors associated with prolonged illness

A systematic review of eight studies examining the frequency of chronic symptoms following IM and associated risk factors was published in 2002.(63) Almost all of the included studies had university students as their participants, and only two of the included studies reported participation and response rates fully. Also, the studies used different reference standard tests to include participants and reported outcomes in a heterogenous manner. A narrative synthesis of the results was provided and concluded that fatigue is the most common persistent symptom after resolution of other acute symptoms of IM, reporting rates of 9-22% of cases at six months following acute illness. The authors also concluded that there was no single psychological or clinical element that was associated with prolonged recovery, but that reduced physical activity was consistently identified as a risk factor (in six of the studies) during the acute and recovery stages. This has also been confirmed in subsequent studies.(214, 215) They noted that the risk factor of reduced activity may be amenable to intervention in primary care. The studies included in the review demonstrated conflicting evidence regarding a link between prolonged fatigue and psychological factors. The review also included studies that examined the effect of antiviral and steroid medication on recovery, and overall found that the medications were ineffective at reducing recovery time

A number of similar studies were published subsequently. A large UK primary care cohort study found that risk factors for fatigue after IM included female sex and premorbid mood disorder.(216) Age was not found to be significantly associated with fatigue following acute IM, although there was a trend for increasing fatigue with increasing age.

An Australian study from 2006 found that a 'post-infective fatigue syndrome' occurred at a comparatively uniform rate (approximately 12%) following acute infection with three different pathogens; EBV, the bacterial pathogen Coxiella burnetii (Q fever) or Ross River virus (epidemic polyarthritis).(33) The fatigue syndrome was generally associated with the severity of the acute illness, rather than microbiological, psychological or demographic influences. The findings imply that it is a factor of the host response to the acute illness, rather than the disease pathogen itself, that determines the risk of developing post infective

fatigue syndrome. Another study from 2006 from the same research group, found that when they examined cases of EBV with prolonged symptoms versus matched cases of EBV with usual recovery, the severity of acute phase symptoms was greater in those with prolonged symptoms.(34) A further paper published by the Australian research group found that 35 genes were abnormally expressed in those patients with prolonged disabling fatigue post EBV IM.(217) The researchers concluded that gene expression may help to explain the pathophysiology of CFS, which is a complex syndrome, or group of syndromes.

A later study of adolescents with IM also concluded that indices of severity of illness (autonomic symptoms and days spent in bed since onset of IM) were the best predictors of developing chronic fatigue syndrome (CFS) following acute IM.(214)

The most recent study in this area was a prospective study of 200 adolescents set in Norway which concluded that development of prolonged fatigue is associated with baseline variables such as negative emotions, verbal memory (delayed recall and recognition) and functional impairments.(215)

Female patients have been shown in several studies to have more severe symptoms and prolonged recovery than males. A Scottish study examining students with IM found that females were more likely to report fatigue, and that the fatigue was significantly more likely to be prolonged (median 118 days versus median 49 days for males in the study).(26) This resulted in females missing more time from their studies, and other activities. A prospective cohort study from the US examining adolescents who developed CFS following IM, also determined that female gender was associated with the development of IM.(208) Another prospective primary care study from the UK found that at six months, fatigue was predicted best by the variables female gender and illness perceptions, and at twelve months by older age and female gender.(218) The link between female sex and fatigue after IM was also seen in the large UK cohort study.(216)

Risk factors associated with delayed recovery following acute IM examined in the studies described above are summarized in Table 7-1 below.

It has yet to be determined whether the syndrome known as ‘long-Covid’ is entirely unique to COVID-19 or whether there is an overlap with other post viral infectious syndromes, e.g., IM, but both conditions have features in common, most notably the symptom of prolonged fatigue.

Table 7-1: Studies examining delayed recovery or fatigue and risk factors following acute IM (adapted from (218))

Study reference	Prevalence of delayed recovery	Fatigue Questionnaire used	Psychological factors	Clinical features	Demographic factors	Behavioural factors
Greenfield et al, 1959 (219) N=38 Retrospective cohort	Information unavailable.	Information unavailable.	Psychological problems at six months associated with self-reported delay.	N/A	N/A	N/A
Dalrymple, 1964 (220) N=131 Quasi-RCT	10% not recovered at 6 weeks.	Information unavailable.	N/A	Clinical features not predictive of poor outcome.	N/A	Bed rest associated with delayed recovery.
Thompson et al, 1969 (221) N=25 Retrospective cohort	56% had fatigue at 6 months, 16% at 11 months.	Information unavailable.	N/A	Splenomegaly at onset predictive of delayed recovery.	No sex difference.	Longer convalescence associated with delayed recovery.
Cadie et al, 1976 (222) N=36 Prospective cohort	At 12 month follow up, 65% of women had depression, 25% had anxiety (pre-IM 25% had depression, 15% anxiety). No difference in men.	N/A	N/A	N/A	Female sex associated with psychological distress in recovery.	N/A
Chretien et al, 1977 (223) N=122 Prospective cohort	Mean duration of illness 28.8 days. Data about delayed recovery unavailable.	Self-reported symptom; 'fatigue'.	N/A	Gastrointestinal symptoms and palatal petechiae associated with delayed recovery.	N/A	Higher number of days from onset to diagnosis best predicted delayed recovery.
Katon et al, 1999 (224) N=144 Prospective cohort	No significant association with anxiety / depression at 6 month follow up.	N/A	More psychological distress at 2 months associated with lower social functioning at illness onset. Distress at 6 months associated with adverse life events.	Distress at 2 months associated with milder symptoms at IM onset.	No association with age / sex.	At six months, distress is associated with reduced activity around onset.
Buchwald et al, 2000	12% failed to recover at 6	Self-reported	More family support and	Higher temperature	Older age was	Poorer physical

Study reference	Prevalence of delayed recovery	Fatigue Questionnaire used	Psychological factors	Clinical features	Demographic factors	Behavioural factors
(32) N=150 Prospective cohort	months.	scale 0-10 (un-named scale).	more life events predictive of self-reported delay in recovery at 6 month follow up. No association found with current psychological disorder.	associated with delayed recovery at 2 months. Other baseline clinical and biological evaluations not associated with delayed recovery.	predictive of delayed recovery at 2 months. Female sex was predictive of delayed recovery at 6 months.	functioning at baseline was predictive of delayed recovery at 2 months.
White, 2001 (62) N=103 Prospective cohort	40% had fatigue, 22% had hypersomnia, 9-22% had CFS at 6 months.	Empirically defined fatigue syndrome (details of measurement instruments not given)	Pre-morbid mood disorder was predictive of fatigue syndrome at 6 months.	Cervical lymphadenopathy was predictive of fatigue syndrome at 2 month. Other clinical features were not associated.	N/A	Physical deconditioning was predicted of fatigue syndrome at 2 months. Physical deconditioning at 2 months predicted CFS at 6 months.
Candy et al, 2003 (218) N=71 Prospective cohort	38% report not being recovered from IM at 6 months, 11% reported fatigue at 6 months.	Chalder Fatigue Questionnaire.	Illness perceptions was a predictor of fatigue at 6 months.	No association found between cortisol levels and fatigue.	Female sex was a predictor of fatigue at 6 months.	No association found between behavioural factors and fatigue.
Petersen et al, 2005 (216) N=1438 Retrospective cohort	12.3% reported fatigue at 2 months. Only 2% received a fatigue diagnosis within a year after IM.	Retrospective study of case notes for fatigue or similar symptoms e.g. 'tiredness, malaise, lethargy, debility.	Premorbid mood disorder was found to be risk factor for fatigue following IM.	No evidence found of association between atopy and fatigue after IM.	Female sex was found to be risk factor for fatigue following IM. Age was not found to be significantly associated, although trend for increasing fatigue with increasing age.	N/A
Hickie et al, 2006 (33)	12% reported fatigue at 6	Used scale that	Psychological factors not	Severity of acute illness	Age and sex not	N/A

Study reference	Prevalence of delayed recovery	Fatigue Questionnaire used	Psychological factors	Clinical features	Demographic factors	Behavioural factors
N=68 Prospective cohort	months. 11% met criteria for CFS at 6 months.	records somatic symptoms (SOMA) to identify clinical features of prolonged fatigue states.	found to be significantly associated with post-infective fatigue syndrome.	was significantly associated with post-infective fatigue syndrome.	found to be significantly associated with post-infective fatigue syndrome.	
Macsween et al, 2010 (26) N=57 Prospective cohort	34% females and 5% males reported fatigue 6 months after acute IM.	Numerical rating scale from 0 to 10, based on the brief fatigue inventory.	N/A	No association found between total number of lymphocytes or viral load and fatigue severity or duration.	Female patients were more likely to have prolonged fatigue and to discontinue studies following IM.	Female patients took less exercise during their illness.
Jason et al, 2014 (214) N=301 Prospective nested case-control	39 participants met criteria for CFS at 6 months.	Autonomic Symptoms Checklist – Patient Version (ASC) and 'checklist of infectious symptoms'.	Psychological factors were not found to be a significant predictor of CFS at 6 months.	Autonomic symptoms at baseline were a significant predictor of CFS at 6 months.		Number of days in bed since IM onset was a significant predictor of CFS at 6 months.
Pedersen et al, 2019 (215) N=200 Prospective cohort	27/195 met criteria for Fukuda definition CFS at 6 months.(210)	Chalder Fatigue Questionnaire (CFQ) and Chronic Fatigue Syndrome (CFS) symptom inventory.	Negative emotions, verbal memory (delayed recall and recognition) and functional impairments associated with higher fatigue score at 6 months.	Higher C reactive protein (CRP) found to be a significant predictor of higher fatigue score at 6 months.	No association was found between female sex and higher fatigue score at 6 months when adjusted analysis was performed.	Higher number of steps per day was found to give lower fatigue scores at 6 months.

7.1.3 Assessment of fatigue

There are a number of tools available to measure fatigue. A 2004 systematic review of fatigue measures concluded that many of the measures used have not been validated, and that it unlikely that any one measure of fatigue would be appropriate for use in all patient populations.(225) A 2009 systematic review examining the performance of fatigue measures also concluded that 'there is no ideal instrument'.(64)

The Fatigue Severity Scale (FSS) (see Appendix 11: Fatigue Severity Scale (FSS)) was developed in 1989 and includes nine questions about the impact of fatigue on elements of daily functioning, which are rated on a seven-point Likert scale.(226) When the scale was developed, the cut-off score for fatigue was deemed to be an average score greater than or equal to four, based on the proportion of patients with medical conditions who reported fatigue above this level.(226) Subsequent studies have also recommended this cut-off score.(227-229) The 2004 and 2009 systematic reviews mentioned above, found that the FSS demonstrates good psychometric properties and demonstrates the ability to detect change over time.(64, 225) Another review of measures of fatigue found that the FSS had excellent reliability and validity.(65) The FSS has been validated in populations with chronic fatigue syndrome but not specifically in populations with fatigue post infectious mononucleosis.

Visual analogue scales are simple psychometric measuring instruments that are used in a wide variety of medical settings for patients to rapidly self-report their symptoms, usually on a scale of zero to ten.(230) The visual analogue scale for fatigue is unvalidated and has some variations (including some more detailed formats with several questions). One of the most commonly used formats asked patients to indicate on number line which score describes their global fatigue (at the moment) with zero being normal and ten being the most fatigued.

Of the fourteen studies summarised in Table 7-1 above, none of them used the FSS or VAS for fatigue to report fatigue scores, and all except two (which used the Chalder Fatigue

Questionnaire) reported fatigue in a heterogenous manner. Many of the studies focussed on CFS or functional activity scales as their primary outcome, rather than fatigue as an individual symptom.

7.1.4 Interventions to improve recovery from IM

Previous studies have shown that reduced activity and illness beliefs may be predictors for prolonged fatigue following IM.(214, 215, 218) A quasi- randomised trial from 1964 examined the effect of restricted activity on recovery from IM.(220) The authors reported that the group allocated to restricted activity (imposed bed rest during the acute phase) took significantly longer to recover than two ambulatory groups, who were advised to continue activities as they wished. An RCT using a brief psychoeducational intervention which aimed to reduce fatigue post IM was piloted in 2004.(231) Although the trial included just 69 patients, the intervention was found to be acceptable and resulted in reduced number of cases of fatigue at six month follow up (OR 0.31, 95% CI 0.09-0.91).

Other studies have demonstrated the positive effect of interventions aimed at increasing physical activity and promoting rehabilitation to accelerate recovery in CFS (not necessarily associated with IM).(232-234)

7.1.5 Relationship between microbiological aetiology during acute illness and outcomes

As mentioned in Section 7.1.2 above, one of the studies examining the relationship between IM and post infective fatigue found that outcomes (in particular fatigue syndrome), were more closely related to the severity of acute illness, rather than the causative microbiological pathogen, when they compared EBV with another viral and bacterial acute illness.(33) This idea has been explored in several more recent similar studies also.

One study examined the differences in outcomes between groups of participants with acute cough caused by bacterial versus viral aetiology (as identified on throat swab).(235) The study (of 645 participants presenting to primary care) found that although there were small

differences in presenting symptoms between viral and bacterial causes, there were no differences in the duration of symptoms between organism groups.

Another recent study investigating this theory was a post-hoc analysis of a large primary care RCT of people presenting with acute influenza like illness.(236) This study again found that there were only minor differences in presenting features between viral and bacterial pathogens, and that the clinical course of disease and risk of complications were not related to the microbiological aetiology.

7.2 Aims and objectives

The aim was to explore potential long-term associations between IM caused by EBV and fatigue and time missed from college / work in younger adults.

7.3 Methodology

The study design was a prospective cohort study, as described in Chapter 3.

7.3.1 Outcome measures

The following outcomes were explored:

- Fatigue:
    i. FSS assessment tool (which gives an average score for fatigue of one to seven, based on seven questions). As recommended in the literature, the FSS score was converted to a dichotomous outcome, using the cut-off score of four and above to denote fatigue (see Section 7.1.3).
    ii. Visual analogue scale (VAS) for global fatigue (which scores fatigue from 0-10). Although it is an unvalidated scale, the VAS for global fatigue was chosen as it provides a quick simple assessment of fatigue (see Appendix 12: Visual Analogue Scale for Global Fatigue).

- Time missed from college / work in number of days, over a three-month period following acute illness.

7.3.2 Explanatory variables

For the exploratory adjusted analysis, three variables were included in the model, EBV diagnosis, sex (based on previous literature in this area), and feeling generally unwell which is self-reported as present or absent at baseline (based on consensus between clinical colleagues who focussed on variables which may indicate a patient being more generally unwell). The variables were previously described in more detail in Section 3.2.4.

7.3.3 Data collection

The baseline and follow up data for the cohort study was collected as described in Section 3.2.3.

7.3.4 Statistical Analysis

7.3.4.1 Descriptive statistics, univariable associations and adjusted analysis for fatigue scores

Initially, appropriate descriptive statistics (mean, standard deviation, mean difference) were used to compare the scores for the two fatigue measures (FSS and VAS for fatigue) in participants with and without a diagnosis of EBV IM.

Missing values for each variable were tabulated. Imputation was not performed. Analysis included only participants with complete data for individual time points.

Fatigue was then examined as a categorical variable (present or absent), in participants with and without a diagnosis of EBV IM at baseline, three months and six months. The cut-off of ≥4 for the FSS is interpreted as 'fatigue present'.

Exploratory analyses were also performed for EBV positive and EBV negative participants examining their fatigue outcomes when adjusted for the variables sex, and 'feeling generally unwell'.

7.3.4.2 Association between time missed from college / work and EBV

Data were initially inspected graphically to examine the potential differences between cases and controls in terms of time missed from college / work. The number of days missed (over a three-month period following acute illness) was categorised into two categories: 0-9 and ≥ 10, to examine those patients who had a prolonged absence of over two working weeks from their usual activities.

All analyses were conducted using Stata version 16.(185)

7.4 Results

Of the 348 participants recruited, 239 (68.7%) submitted complete follow up data at three months, and 196 (56.3%) submitted complete follow up data at six months. Figure 7-1 demonstrates the response rates for the fatigue scores follow up data at three and six months (overall proportions for EBV positive and negative at baseline were 12.8% and 87.2% respectively). The response rates at follow up were slightly higher for EBV positive when compared to EBV negative participants. As a results of this missing data due to follow-up losses, and given the small number of EBV positive participants at 3 and 6 months time points, this work is exploratory and results should be interpreted with caution.

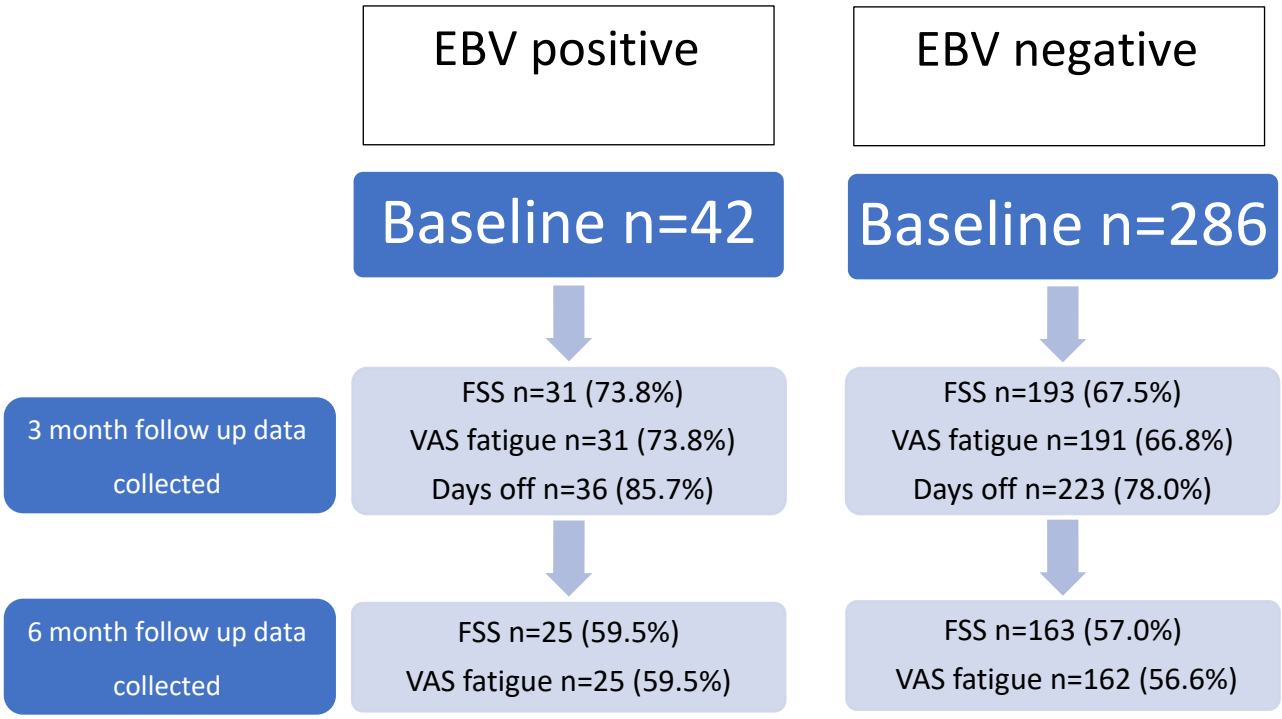

Figure 7-1: Flowchart for participants and follow up response rates

7.4.1 Descriptive statistics, univariable associations and adjusted analysis for fatigue scores

Table 7-2 compares the self-reported fatigue scores, in participants with and without a diagnosis of EBV IM. FSS is scored from 1 to 7, with a cut-off score of 4 for interpreting the FSS as 'fatigue present'. VAS for fatigue is scored from 0-10. Overall, the fatigue scores decreased as time went on. Of note, overall, both fatigue scores were consistently higher at all time points in the EBV positive group, with the mean score for FSS at the six-month follow-up point being 3.9, which is just below the cut-off for 'fatigue present'. The mean difference between groups for FSS at baseline was 0.12, at three months was 2.8 and at six months was 1.05, with higher scores in the EBV positive group. The mean difference between groups for VAS for fatigue at baseline was 0.35, at three months was 0.31 and at six months was 0.14, with higher scores in the EBV positive group.

Table 7-2: Descriptive statistics of outcomes at 3 and 6 months

	EBV positive Mean (SD)	EBV negative Mean (SD)	Mean difference between groups (95% CI)
FSS Baseline (n=328)	4.3 (1.2)	4.2 (1.3)	-0.12 (-0.56 to 0.31)
3 months (n=224)	4.2 (1.5)	3.9 (1.3)	-2.80 (-7.20 to 1.61) n=184
6 months (n=188)	3.9 (1.5)	3.7 (1.3)	-1.05 (-6.12 to 4.01) n=157
VAS for fatigue Baseline (n=328)	5.9 (2.1)	5.6 (2.0)	-0.35 (-1.05 to 0.34)
3 months (n=222)	4.1 (2.7)	3.8 (2.6)	-0.31 (-1.29 to 0.67) n=189
6 months (n=187)	3.8 (3.0)	3.6 (2.6)	-0.14 (-1.26 to 0.99) n=160

The odds ratio for having fatigue in the EBV positive versus negative groups was 1.11 (95% CI: 0.54-2.26) at baseline increasing to 1.70 (95% CI: 0.78-3.70) at three months, and 1.73 (95% CI: 0.74-4.05) at six months. However, there was no evidence of a statistical association between EBV and fatigue.

Table 7-3 examines fatigue (presented as a categorical variable; present / absent) in participants with and without a diagnosis of EBV IM at baseline, three months and six months. The cut-off of ≥ 4 for the FSS is interpreted as 'fatigue present'. The total number of participants was 309 at baseline, 224 at three months and 188 at six months. In summary, the proportion of participants with FSS ≥ 4 was higher in the EBV group at all time points, with over half of cases of EBV (56%) self-reporting fatigue at six month follow up. However, over 40% of EBV negative patients also reported fatigue at six month follow up. The odds

ratio for having fatigue in the EBV positive versus negative groups was 1.11 (95% CI: 0.54-2.26) at baseline increasing to 1.70 (95% CI: 0.78-3.70) at three months, and 1.73 (95% CI: 0.74-4.05) at six months. However, there was no evidence of a statistical association between EBV and fatigue.

Table 7-3: proportion with fatigue (presented as a categorical variable) by EBV positivity

	EBV positive N (%)	EBV negative N (%)	OR for fatigue in EBV positive versus negative (95% CI)
Fatigue present (FSS ≥ 4) at baseline	22 (61.1)	160 (58.6)	1.11 (0.54-2.26)
Fatigue present (FSS ≥ 4) at 3 months	19 (61.3)	93 (48.2)	1.70 (0.78-3.70)
Fatigue present (FSS ≥ 4) at 6 months	14 (56.0)	69 (42.3)	1.73 (0.74-4.05)

Analyses were also performed for EBV positive and EBV negative participants examining their fatigue outcomes when adjusted for the variables sex and 'feeling generally unwell'. The results did not show any statistically significant associations between EBV diagnosis and FSS or VAS at three and six months (Table 7-4).

Table 7-4: Adjusted linear (FSS) and logistic (VAS for fatigue) regression analyses exploring the potential association between EBV and fatigue

FSS	**OR (95% CI)***	**p-value**
3 months	1.96 (0.81 to 4.73)	0.13
6 months	2.16 (0.83 to 5.62)	0.12
VAS for fatigue	**β(95% CI)***	**p-value**
3 months	0.23 (-0.75 to 1.21)	0.64

6 months	0.10 (-1.00 to 1.20)	0.85

*All models adjusted for sex and feeling generally unwell

7.4.2 Association between time missed from college / work and EBV

The mean number of days missed from college / work for EBV positive participants was 6.39 (SD 6.85). The mean number of days missed from college / work for EBV negative participants was 3.86 (SD 4.25). The overall mean in the whole study population was 4.21 (SD 4.77). The median number of days missed was 3 days, and the mode was 0 days, in both EBV positive and negative groups. The interquartile range (IQR) for the EBV positive group was 6.5, and for the EBV negative group was 4.

Figure 7-2 demonstrates the total number of days missing from college / work for EBV positive versus EBV negative.

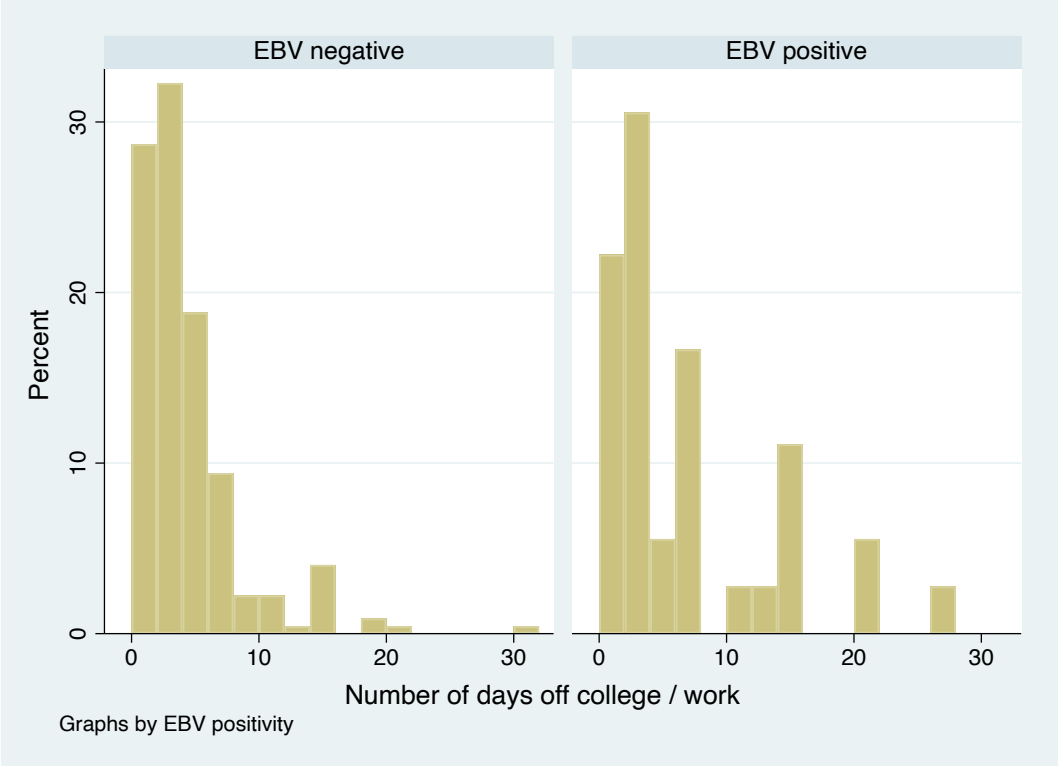

Figure 7-2: Total number of days off by EBV positivity

The maximum number of days missed was 28 in the EBV positive group, and 32 in the EBV negative group.

Given the skewed nature of the data, we choose to dichotomise the number of days missed into two categories; 0-9 days and ≥ 10 days.Table 7-5 demonstrates the self-reported number of days missed from college / work over the initial three month follow up period, when categorised into two groups; 0-9 days and ≥ 10 days. 75% of EBV positive and 92% of EBV negative patients missed less than ten days, whereas 25% of EBV positive and only 9% of EBV negative patients missed ten or more days. The odds ratio for missing ten or more days (compared to 0-9 days) for EBV positive versus EBV negative patients was 3.58 (95% CI 1.47 to 8.71; p<0.01). Table 7-5: Number of days off college / work by EBV positivity

Total number of days off college / work over 3-month period	EBV positive N=36 n(%)	EBV negative N=223 n(%)	Total N=259 n(%)
0-9	27 (75)	204 (91.5)	231 (89.2)
≥ 10	9 (25)	19 (8.5)	28 (10.8)

7.5 Discussion

7.5.1 Main Results

Only 56% of participants completed follow up questionnaires at the six month follow up point. At three months, 19/31 (61.3%) respondents who were EBV positive had fatigue, and at six months 14/25 (56%) of respondents who were EBV positive had fatigue.

There was a small mean difference between groups for FSS and VAS for fatigue at all time points (higher in the EBV group) but with wide confidence intervals. The proportion of participants with self-reported fatigue (i.e. FSS ≥ 4) was slighter higher in the EBV group at all time points. The results demonstrated a small, non-significant mean difference between EBV positive and EBV negative groups, but again, with wide confidence intervals. The clinical importance of these mean differences in fatigue are of questionable clinical significance.

Adjusted analyses were conducted to explore potential associations between fatigue outcomes and EBV adjusting for the variables sex and 'feeling generally unwell'. The results do not show any statistically significant associations for FSS or VAS at follow up time points.

Cases of EBV also had a higher proportion of more time missed from usual activities in the first three months following acute IM, when compared with those who had negative results for EBV. The odds ratio for missing ten or more days (i.e. two working weeks) for EBV positive versus EBV negative patients gave a statistically significant result of 3.58 (95% CI 1.47 to 8.71; $p<0.01$). So whilst fatigue symptoms remained relatively common at follow up for both EBV positive and negative participants, there was no statistically significant difference between the two groups, reflecting low power because of substantial losses to follow up.

7.5.2 Context of other studies

This study with participants recruited from student health centres described very high rates of self-reported fatigue in both the EBV positive and EBV negative patients at six months. It is difficult to compare the results with those of previous similar studies as they all use different measures of fatigue and often have CFS or functional criteria rather than fatigue itself as their primary outcome. Most of the studies report the proportion of participants who had failure to recover or were diagnosed with CFS by a certain time point, rather than reporting odds ratios or mean difference between participant groups. The 2002 systematic review that examined the frequency of chronic symptoms following IM could provide only a narrative synthesis due to the heterogeneity of the eight included studies.(63) They reported that that fatigue was present at rates of 9-22% of cases at six months following acute IM. It is plausible that a student population may self-report higher rates of fatigue than a general primary care population, however, most of the studies in the systematic review had participants recruited from student health centres, similarly to this study, and the rates of fatigue are still very disparate.

It is possible that because this study had participants with sore throat from another cause (e.g., other viral causes and GABHS), that their higher rate of fatigue at follow up could be accounted for by post infectious fatigue (from other microbiological pathogens), thus the rate is higher than that reported in studies using healthy controls as a comparison group. As referred to in Section 7.1.5, literature has been published in this area, specifically in relation to EBV and other pathogens, but also in relation to outcomes from other viral and bacterial causes of acute respiratory illness.

Previous studies generally show associations between IM and prolonged fatigue, and between female sex and prolonged fatigue after IM.(26, 208, 218) The association between fatigue and female gender was borne out in this study in the adjusted analyses for the VAS for fatigue outcome at three-month and six-month time points, but not for the FSS.

7.5.3 Clinical value and implications for practice

The results of this study are based on very small numbers, and are underpowered to show any definitive findings. The results should be interpreted with caution, in particular the rates of fatigue at six months, which were dissimilar to previous study findings. It is, however, interesting to note the possible association between female sex and prolonged fatigue (as reported in previous literature), and this may be an area to target interventions to reduce morbidity from IM, using for example, graded activity and lifestyle management.(220, 231) A small pilot study from 2004 using a brief psychoeducational intervention post IM found reduced rates of fatigue at six month follow up, and studies of interventions for CFS have also found a positive effect from increasing physical activity.(231-234)

7.5.4 Future research

Future research should focus on large prospective studies with enough power to accurately examine length of recovery following acute IM, all possible predictors that influence it, and which interventions may aid in recovery. One possible research design would be to conduct a prospective study large enough to derive and internally validate a CPR for post infectious

fatigue following acute sore throat, possibly incorporating various microbiological aetiologies in different groups.

7.5.5 Strengths and limitations of study

A major strength of this study was that it was a prospective cohort study of IM caused by EBV, based in student health primary care centres, of which there are few studies. Most previous cohort studies performed examining the natural history of IM are older or smaller studies; some have not been performed prospectively, and most of them do not have a comparison or control group.

In this study of young adults, we have compared the outcomes of participants diagnosed with IM caused by EBV with a comparison group of those participants who present with similar acute symptoms to cases but who were not diagnosed with EBV IM. Most of the comparison participants have an upper respiratory tract infection as their differential diagnosis and it is interesting to examine their outcomes in comparison to the cases, rather than comparing with healthy controls.

A limitation of this study is that the sample size was small and there was a high rate of participants lost to follow up (attrition bias), which means all results need to be interpreted with caution. Just over half (56%) of participants submitted follow up data at six months, and those with persistent symptoms may have been more likely to contribute, which could have resulted in overestimating the number of participants in both groups who still reported fatigue. However, both EBV positive and EBV negative participants completed similar rates of follow up, so potentially the effect of attrition bias on the differences between EBV and non-EBV groups will not have been that large.

Furthermore, follow up outcomes were of subjective and self-reported measures. Participants did not know their diagnosis at baseline, when they had measures taken for fatigue scores, however, knowing their diagnosis at three and six month follow up may potentially bias them into reporting higher scores. There is no universally used measure for

fatigue, and the fatigue scores chosen, although one was validated, did not seem to differentiate well between those with and without fatigue. It is surprising that over 40% of the non EBV group reported fatigue being present at six month follow up. A more useful score might be the Chalder Fatigue Questionnaire, which has been used in two previous similar studies.(215, 218)

This study recruited participants from a student health population, which may mean that the results are not readily generalisable to other primary care settings.

7.6 Conclusion

This study found substantial morbidity in patients, with a large number of those in both EBV and non-EBV groups (56% and 42% respectively) still reporting fatigue six months following acute illness. This is much higher than rates reported in other cohort studies examining fatigue following IM (9-22%), and also much higher in comparison to a large UK general population study, in which in 18% of people reported fatigue being present for more than six months.(32, 33, 63, 208, 209, 212) Consistent with the other literature in this area, female patients were more likely to report higher rates of fatigue. Cases of EBV missed significantly more time from college or work than those who were negative for EBV. The odds ratio for missing ten or more days for EBV positive versus EBV negative patients was 3.58 (95% CI 1.47 to 8.71).

Unfortunately, due to inadequate sample size, results reported in this study should be viewed as exploratory and need to be interpreted with caution.

Strategies to reduce long term morbidity of IM, especially in terms of prolonged fatigue, would be useful, and may be amenable to implementation in primary care.

8 Chapter 8: Overall Discussion

This discussion chapter will present an overview of the thesis main findings for each aim, a consideration of the strengths and limitations of the thesis, and how this research informs clinical practice and future research.

The thesis aimed to further research in the area of improving the diagnosis and management of sore throat, especially sore throat caused by IM. There were four broad objectives;

I. To systematically review the effectiveness of antiviral therapy for infectious mononucleosis (IM) (this was originally completed and published in the Cochrane Database of Systematic Reviews; with searches updated for the thesis).(144)
II. To derive and externally validate a clinical prediction rule (CPR) to aid in the positive diagnosis of IM caused by EBV.
III. To validate the Centor score CPR in an Irish setting.
IV. To explore potential associations between IM caused by EBV and fatigue and time missed from college / work in younger adults.

8.1 Systematic review of the effectiveness of antiviral therapy for infectious mononucleosis

Chapter 2 presents the results of this systematic review. Seven RCTs with a total of 333 participants were included in the review. Three trials studied hospitalised patients, two trials were conducted in an outpatient setting, while the trial setting was unclear in two studies. Participants' ages ranged from two years to young adults. The type of antiviral, administration route, and treatment duration varied between the trials. The antivirals in the included studies were acyclovir, valomaciclovir and valacyclovir. Follow-up varied from 20 days to six months. The diagnosis of IM was based on clinical symptoms and laboratory parameters.

The risk of bias for all included studies was either unclear or high risk of bias. The quality of evidence was graded as very low for all outcomes and so the results should be interpreted with caution. There were statistically significant improvements in the treatment group for two of the 12 outcomes. These improvements may be of limited clinical significance.

There was a mean reduction in 'time to clinical recovery as assessed by physician' of five days in the treatment group but with wide confidence intervals (CIs) (95% CI -8.04 to -1.08; two studies, 87 participants). Prospective studies indicate that clinical signs and symptoms may take one month or more to resolve and that fatigue may be persistent in approximately 10% of patients at six-month follow-up, so this may not be a clinically meaningful result.

Trial results for the outcome 'adverse events and side effects of medication' were reported narratively in only five studies. In some reports authors were unsure whether an adverse event was related to medication or complication of disease. These results could not be pooled due to the potential for double counting results but overall, the majority of trials reporting this outcome did not find any significant difference between treatment and control groups.

There was a mean reduction in 'duration of lymphadenopathy' of nine days (95% CI -11.75 to -6.14, two studies, 61 participants) in favour of the treatment group.

In terms of viral shedding, the overall effect from six studies was that viral shedding was suppressed while on antiviral treatment, but this effect was not sustained when treatment stopped.

For all other outcomes there was no statistically significant difference between antiviral treatment and control groups.

Overall, the systematic review demonstrated that effectiveness of antiviral agents (acyclovir, valomaciclovir and valacyclovir) in acute infectious mononucleosis is uncertain. The quality of the evidence is very low; included studies were small, heterogeneous and at unclear or

high risk of bias. Outcomes were selectively reported, often reported as composite scores and generally found only modest improvements which may not be clinically meaningful.

Two ongoing RCTs were found in the updated December 2021 search.(166, 167) Both trials appear to be publicly funded. The trial set in China includes over 600 participants, while the trial set in Iran includes just 46. The trial set in China did not have easily accessible information study methods, other than stating that it is an RCT. The trial set in Iran reported that the design was a double-blinded RCT. More detailed information would need to be obtained about the methods of these trials; risk of bias etc., before their results could be deemed suitable for inclusion into an update of this systematic review.

8.2 Derivation and external validation of a clinical prediction rule (CPR) to aid in the positive diagnosis of IM caused by EBV

Chapters 4 and 5 present the derivation, internal validation and external validation of a CPR to aid in the diagnosis of IM caused by EBV. Though there has been a systematic review examining the signs and symptoms most associated with a positive diagnosis of IM, there has not been a CPR developed for this purpose previously.(40)

Four models were developed, each of which had individual strengths and weaknesses, but gave similar results for calibration and discrimination. Development of the CPR was limited by having a low number of participants who tested positive, but the CPR performed reasonably well in internal and external validation despite this. The internal validation gave results for discrimination for Model 4, as measured by AUC, which was reasonable (AUC: 0.70; 95% CI: (0.62 – 0.79)) but calibration was good (Hosmer-Lemeshow goodness-of-fit: χ^2 = 0.7315; p=0.63). Models 1-4 varied in their maximum post-test probability; scoring 61.9%, 43.4%, 52.2% and 23.2% respectively. Using these results in conjunction with the variable atypical lymphocytosis, produced a post-test probability of >80% (with relatively narrow confidence intervals) for all four models, which demonstrates the highly predictive nature of this variable.

Model 4 was chosen to be externally validated. This was for pragmatic reasons, as collaboration with international colleagues allowed validation of their dataset, which included data on both variables (presence of exudate on pharynx and presence of enlarged / tender posterior cervical lymph nodes) in Model 4 but did not include data needed to validate the other models. This external validation in a separate population indicated that CPR Model 4 had reasonable discrimination and good calibration . The AUC was 0.69 (95% CI: 0.67 to 0.72) indicating reasonable discrimination, and the Hosmer-Lemeshow goodness-of-fit showed no evidence of a significant difference between the expected and the observed risk of EBV ($\chi^2 = 3.40$; $p = 0.183$), indicating good calibration.

Using the validated two variable CPR for IM, if a young adult patient presents with sore throat and has the two predictive factors in the model, this increases their chance of EBV IM diagnosis to 33%-52% (with relatively wide confidence intervals), depending on their pre-test probability rate. If one or both of the variables from the CPR is present, the patient may be categorised as intermediate risk of EBV IM diagnosis, and clinician may be more inclined to perform a diagnostic test (either FBC as a triage test, or EBV serology testing), whereas if neither variable is present, the patient may be categorised as low risk, allowing the clinician to adopt an expectant management approach.

8.3 Validation of the Centor score CPR in an Irish setting

Chapter 6 presents the results of validating the Centor score CPR in an Irish student health primary care setting.(74) Sore throat / sore throat is one of the most common presentations in primary care, especially in young adults. Most cases are caused by viruses, but in approximately 10-15% of adult cases GABHS is the cause.(3, 7)

There are many CPRs in use for the diagnosis of GABHS sore throat, but the Centor score is longest established and is one of the most widely used. The score includes four variables: tonsillar exudates, swollen tender anterior cervical lymph nodes, absence of cough and history of fever. The CPR was developed to help clinicians distinguish between viral sore throat and sore throat caused by GABHS, and thus guide appropriate management. This is

especially relevant in terms of which patients may need to have a throat swab to confirm diagnosis, and whether antibiotics are indicated or not. The CPR has undergone many external validations; however, it has never been validated in an Irish primary care setting before.

The rate of positivity for GABHS in this study population was 23.4%, which is higher than the rates found in the existing literature, and may reflect carriage of GABHS, which is known to be higher in younger populations. The rate of concomitant GABHS and EBV in this study population was 1.5%, which is similar but slightly lower than rates of 2-4% found in previous studies.(103-105) It was also similar to rates of up to 2.3% reported for asymptomatic GABHS carriage.(106-108)

When the original Centor score was validated in this study population, it was found to have poor discrimination (with AUC of 0.55) but the Hosmer-Lemeshow goodness-of-fit showed no evidence of a significant difference between the expected and the observed risk of GABHS ($\chi^2 = 2.60$; $p = 0.273$), indicating good calibration. Likelihood ratios were calculated to be between 1 and 1.3 indicating minimal increase in the likelihood of GABHS sore throat as the Centor score increased. For example, having a score of 4 or above using the CPR, the probability of having GABHS sore throat increased just over 3% from 15.0% (which was the pre-test probability) to 18.3%.

Variables in the Centor score were also tested to investigate if they had an effect that was clearly different in the validation sample. Likelihood ratio tests of the model updates showed evidence ($p<0.05$) that history of fever should be omitted, and absence of cough increased to 2 points. The AUC still indicated poor discrimination, with AUC of 0.59. Again, the Hosmer-Lemeshow goodness-of-fit indicated very good calibration.

Finally, additional variables were included in an extended model to explore whether their addition improved the predictive value of the model. There was no evidence of an effect for age, pulse and feeling generally unwell, however, clinician impression did have a significant effect in improving the performance of the CPR.

Having updated and extended the Centor Score, the final model included tonsillar exudates (1 point (pt)), tender enlarged cervical lymph nodes (1 pt), absence of cough (2 pt) and clinical Impression (1 pt). Discrimination was still poor (AUC: 0.62) but calibration was good. All LR values were calculated at between 1 and 2 indicating minimal increase in the likelihood of GABHS sore throat, for example, having a score of 4 or above using the clinical prediction rule, the probability of having GABHS sore throat increased just over 5% from 15.0% to 20.8%.

A final analysis was performed to examine how the original Centor score performed in an expanded study population including participants with other bacterial causes of sore throat, i.e., Group C / G Streptococcus and fusobacterium positive. The results again indicated poor discrimination but good calibration.

8.4 Observational study to examine symptom duration and complications of IM

Approximately 9-22% of people who are diagnosed with IM report fatigue as being present at six months subsequently.(32, 33, 63, 208, 209) Reduced physical activity was identified (in a systematic review of eight studies), as a risk factor during the acute and recovery stages and this was confirmed in subsequent studies.(63, 214, 215) The literature in this area also demonstrates that female patients generally have more severe symptoms and prolonged recovery from IM than males.(26, 208, 216, 218) However, many of the cohort studies in this area are outdated, and used heterogenous methods for diagnosis of IM and for measuring fatigue outcomes.

In this prospective study of young adults with sore throat, we compared the outcomes of participants diagnosed with IM caused by EBV with a comparison group of participants who presented to their GP with sore throat, but who were not diagnosed with EBV IM.

Response rates at three month follow up were 68.7% and at six month follow up were just 56.3%, and rates were similar in EBV positive and EBV negative participants. Because of the study being underpowered, any results are purely exploratory.

56% of EBV positive participants and 42% of EBV negative participants reported fatigue at six month follow up. There was a small mean difference between groups for FSS and VAS for fatigue at all time points (higher in the EBV group) and similarly for self-reported fatigue (i.e. FSS ≥ 4) but with wide confidence intervals for both analyses. The odds ratio for having fatigue (FSS ≥ 4) in the EBV positive versus negative groups was slightly greater than 1 at baseline increasing to 1.70 at three months, and 1.73 at six months.

Consistent with the literature in this area, adjusted analyses demonstrated that female gender was found to have a small positive statistically significant effect on VAS for fatigue at three and six months follow up but not on FSS.

Cases of EBV also had a higher proportion of more time missed from usual activities in the first three months following acute IM, when compared with those who had negative results for EBV. The odds ratio for missing ten or more days (i.e. two working weeks) for EBV positive versus EBV negative patients gave a statistically significant result of 3.58.

Interventions have been shown in previous literature to be effective for reducing delayed recovery in IM and may be amenable to application in primary care.(220, 231)

Whilst it is important to have a positive diagnosis of IM for the purposes of avoiding unnecessary antibiotics, and awareness of potential serious complications, (including the need for important advice about the avoidance of contact sports) there is less certainty about a specific link between IM and persistent fatigue. This research adds to the literature in this area that indicates that finding the specific microbiological cause of acute respiratory illness may not have a substantial impact on long term outcomes, i.e. all pathogens can lead to persistent fatigue in some individuals, and underlying mechanisms are not understood.

The growing population of people who have recovered from acute Covid-19 provides researchers with the opportunity to better understand the concept of ‘long Covid’, and this in turn may help to shed light on other post viral syndromes.(237)

8.5 Strengths and limitations of this thesis

Overall, this thesis addressed a clinically important and challenging area of primary care by utilising a 2.5-year prospective cohort. The cohort described in this thesis differs from other cohort studies in that we have studied two different conditions, prospectively in a young healthy primary care population. This study is novel in developing and externally validating a CPR for IM in geographically distinct populations. It is also the first time the Centor Score CPR has been validated in an Irish primary care setting. The study design methodologies and reporting were carried out in accordance with best practice guidelines; Cochrane / PRISMA, TRIPOD and STROBE.(159, 238-240)

There were a number of limitations, however. Firstly, the target sample size for two of the studies (derivation of CPR for IM, and validation of Centor Score CPR) was not reached, so it is difficult to draw definite conclusions about the results and their external validity.

The thesis only examined infectious mononucleosis (IM) caused by EBV. Although the findings will be applicable to approximately 90% of cases of IM, it will not be relevant for all, as the causative agent may be different in other cases. It was necessary, however, to focus on EBV for the purposes of this study as testing for and diagnosis of all pathogens was not feasible.

The study may be open to spectrum bias when considering external validity for other settings, because participants were recruited from student health centres where we would expect participants to be relatively young and healthy prior to their current illness and may not be readily generalizable to other settings e.g., hospitalised patients or children. However, this setting has been studied most frequently in the literature to date as it is a group that has a higher incidence of IM, and it is representative of the population we are trying to study.

The derivation and validation cohorts for the CPR for IM differed regarding their inclusion criteria, and also the diagnostic test used. There may have been participants included in the Centor CPR validation study that were actually GABHS carriers rather than having acute

GABHS but were assumed to be true positives for GABHS since they were symptomatic with sore throat.

In terms of the cohort observational study, there was a drop-out rate of almost 44% at the six month follow up point, and participants with persistent symptoms may have been more likely to complete follow up questionnaires, which would have resulted in overestimating the number of participants who still reported fatigue at six months post-acute infection. Using self-reported fatigue scores for follow up of patients in the cohort study may have potentially biased them into reporting higher scores, however patients could not be blinded to their diagnosis.

8.6 Clinical implications

The findings of this thesis, integrated with current evidence, have implications for sore throat consultations in primary care. The clinical implications will be considered under the headings of the four overall objectives.

8.6.1 Systematic review of the effectiveness of antiviral therapy for infectious mononucleosis(IM)

The systematic review examining the effectiveness of antiviral agents in acute infectious mononucleosis concluded that the effect is uncertain, due to very low-quality evidence. Only modest improvements were reported in the outcomes, which may not be clinically important. Alongside the lack of evidence of effectiveness, decision makers need to consider the potential adverse events and possible associated costs, and antiviral resistance. It may be necessary to update this systematic review when the results of the two ongoing RCTs become available, depending on whether they meet inclusion criteria in terms of study design. Because IM has relatively low incidence and even lower frequency of complications, it is difficult to design trials that examine outcomes such as serious complications and death. It's possible that the study of antivirals for IM may be informed by our broader knowledge of antivirals for other conditions.

A recent RCT of an antiviral agent (oseltamivir) for influenza like illness found that the antiviral reduced duration of symptoms by approximately one day overall, but by up to three days in higher risk groups (older patients with more severe illness and comorbidities).(241) This, in addition to the huge resources currently being invested in the area of antivirals for treating Covid-19, may hasten the development of antivirals targeting EBV.

8.6.2 Derivation and external validation of a clinical prediction rule (CPR) to aid in the positive diagnosis of IM caused by EBV.

Although the CPR for diagnosis of IM caused by EBV demonstrates moderate rather than high discrimination, it may help clinicians stratify patients into low, intermediate and high-risk groups, or even into low and intermediate groups (since there is no specific treatment for IM), especially when used in series applying the likelihood ratios for atypical lymphocytosis. In terms of clinical decision making, application of a CPR is likely to be most useful in performing an important 'rule out' function. There are no pre-defined test / treat thresholds for IM, unlike some other conditions e.g. influenza, acute coronary syndrome and community acquired pneumonia (CAP).(127, 143) However, if neither CPR variable is present in a patient, especially if the patient's age is under 15 or over 25 (at which point the pre-test probability drops to approximately 2% or less), the clinician may be reasonably satisfied that the post-test probability is low enough for them to manage the patient expectantly, or consider an alternative diagnosis.(6) The likelihood ratios from having both variables in the CPR are doubtless too low (post-test probability at 23.2% for the age range 16-20 years, and lower at other ages) for a clinician to diagnose IM caused by EBV without first ordering a triage or definitive serology test.

FBC may be useful as a triage test, to rationalise the use of a test for EBV serology.(120) The FBC is used to check specifically for a high proportion of lymphocytes, and in particular, atypical lymphocytes. The thesis findings showed that a high proportion of atypical lymphocytes was strongly associated with a diagnosis of IM. The existing literature has

shown that a combination of >50% lymphocytes and > 10% atypical lymphocytes has a specificity of 0.99 but a sensitivity of 0.45 for the diagnosis of IM, which means there is a high false negative rate, but given the high specificity, if positive, and in the presence of suggestive clinical findings, it could be considered diagnostic.(40) The diagnostic sequence would be to use the CPR based on symptoms and signs, and then test for FBC in intermediate probability cases. Laboratory testing in Ireland for FBC costs approximately 14 euro, while testing for EBV serology costs approximately 20 euro. The laboratory turnaround time for an FBC test is maximum one working day, while it is maximum three working days for EBV serology. Usually if the lymphocytes show atypical morphology, the laboratory report includes comment to that effect and usually suggests testing for IM. In an Irish general practice context, the savings in cost in ordering an FBC versus EBV serology is potentially not worth the possibility of false-negative results, which may result in misinformation around recovery and in particular avoidance of contact sports in a generally young active population. However, if it is used as a 'rule-in' test (as is more suitable due to its higher specificity), this is not such an issue. FBC also has the potential to give information about alternative differential diagnoses, or to differentiate viral from bacterial aetiology more generally. However, if the FBC does not show indications of IM, but the patient has symptoms and signs which are clinically highly suggestive, a definitive test would be required, which adds to practice workload along with inconveniencing and causing potentially additional expense to the patient. FBC is not currently available as a POCT in Ireland but is in other countries. If FBC was readily available as a POCT it may be helpful to use it in conjunction with the CPR to triage which patients need to have a definitive test for IM.

There is scant literature about EBV POCT / rapid antigen testing. At present, it has not been developed enough to the extent where it can give an accurate result and a high rate of false positive results is an issue.(242, 243) Given the extensive use and acceptability of rapid antigen tests for COVID-19 by patients and physicians alike, it may follow that rapid antigen testing for other viruses will be developed in the near future. However, COVID-19 has highlighted potential pitfalls of indiscriminate use of antigen tests when community prevalence is low. If more accurate rapid antigen testing for EBV became more widely available, this could be used in conjunction with the CPR.

Although it is simple (Model 4 included two variables; presence of enlarged / tender posterior cervical lymph nodes weighted with 5 points, and presence of exudate on pharynx weighted with 3 points), the point scoring method for the CPR may be difficult to remember and would be supported by incorporating it into computer based clinical decision support systems (CDSSs) and into GP software systems to facilitate application at the point of patient care.

8.6.3 Validation of the Centor score CPR in an Irish setting

A 2011 systematic review of 21 studies examining the diagnostic accuracy of symptoms and signs and validation of the Centor score found that although the Centor score CPR was well calibrated for use in different settings, it should be used with caution because of the low prevalence of GABHS in a primary care setting.(198) A subsequent large-scale validation of the Centor score was performed in a USA retail health chain population in 2012.(200) This study included >200,000 patients aged over 3 years old, who presented with sore throat to a retail health clinical setting. Although the validation population differed from the derivation population (retail health v's emergency department setting), the discrimination of the CPR was found to be good at 0.72 for patients aged 15 or older.

Our study differed in that discrimination was poor for validation of the original Centor score, and also for its update and extension. An explanation may be that the CPR did not perform as well as in previous validation studies due to spectrum bias in our relatively homogenous, small study population with high rate of GABHS positivity on throat swab (some of which almost certainly is due to carriage).(188) Also, our study population appeared to have less severe illness than the original derivation population, implied by a lower proportion of cases with a Centor score of 4, and the fact that they were recruited from a primary care setting rather than an emergency department (as in the original population).

It is possible that using one of the variations of the Centor score, perhaps incorporating duration of illness and age would have resulted in a better performance in our study population.

The literature demonstrates that there is a direct link between attendance rates for primary care physicians and antibiotic prescribing, and that the threshold to interact with a GP is lowered by telemedicine.(244-246) With the advent of the Covid-19 pandemic, increasing use of telemedicine, and huge pressures on all aspects of the health service, clinicians should be mindful of the risks of overprescribing of antibiotics.

In terms of guidelines for the diagnosis and management GABHS, and in light of increasing antimicrobial resistance, it seems prudent to adopt the guidelines of the HSE, NICE and SIGN, incorporating a CPR (rather than throat swab or RADT) to aid in diagnosis, and reduce antibiotic prescribing. It is likely that most patients would benefit more from sensible self-management advice, with safety netting systems in place in case of deterioration. The literature demonstrates that antibiotics are of minimal benefit in sore throat, even when GABHS is present, but it is difficult not to prescribe an antibiotic to a patient when both clinician and patient know that their test result is positive.(87)

8.6.4 Exploration of potential associations between IM caused by EBV and fatigue and time missed from college / work in younger adults

Although the sample size was small, and the results may not be clinically meaningful, this cohort study contributes to the literature in this area. It may be useful when speaking with patients who have been diagnosed with IM about their prognosis, especially in terms of time missed from usual activities and self-management. On the basis of this study, fatigue rates following presentation with acute sore throat were only slightly higher in those who were EBV positive, although the EBV positive cases did miss more time from college / work, with a mean of 6.39 days. The odds ratio for missing ten or more days for EBV positive versus EBV negative patients was 3.58 (95% CI 1.47 to 8.71). Self-management advice should be given to patients, about avoiding prolonged bed rest and trying to stay active as

much as possible, while avoiding certain high risk activities like contact sports for the first eight weeks.

8.7 Research implications

The findings of this thesis highlight that further research is required in a number of areas.

In terms of the question of whether antivirals are effective in the treatment of IM, more robust clinical studies are required to further assess this research question as the quality of the current evidence is poor and based on small heterogenous studies. Most cases of symptomatic IM are encountered in young adults in a primary care environment, often in student health centres. Trials of commonly used orally administered antivirals versus usual care should be established in this setting. Outcomes examined should include effectiveness on acute symptoms, adverse effects, time off work or school, prevention of complications, effect of antivirals on longer-term outcomes such as fatigue, and evaluation of economic outcomes. The two ongoing studies in this area should be assessed for suitability for inclusion to an update of this systematic review when their results are available.(166, 167)

The CPR for the diagnosis of IM caused by EBV should undergo further prospective validation in diverse clinical settings before it is deemed suitable for widespread use. Additional research is required to develop test and treatment thresholds for IM so that definitive decisions can be made by treating clinicians when using the CPR.

The Centor score CPR for the diagnosis of GABHS sore throat performed poorly in our validation cohort, despite previous studies reporting better performance in large scale validation studies. The FeverPAIN score CPR is used in Irish, UK and Scottish guidelines on the management of sore throat, however, it hasn't been externally validated to date.(180) Future research should focus on large prospective primary care studies to external validation of the FeverPAIN score to assess whether it is suitable for use as a decision aid in primary care patients presenting with sore throat.

Future research should focus on to accurately examine length of recovery following acute IM, the factors that influence it, and which strategies may aid in recovery. Such a prospective cohort study could easily be facilitated by primary care electronic records, flagging an initial acute diagnosis of IM, and then scheduling prompts to ask the patient about fatigue or other symptoms at follow up time points. The recent research in relation to MS and EBV may hasten development of a vaccine in this area.

8.8 Conclusions

The presentation of sore throat is a very common one in primary care. This thesis adds to the literature in this area by examining its diagnosis and management in young adults, especially focussing on two conditions; IM caused by EBV and sore throat caused by GABHS. This thesis describes tools that can aid in the diagnostic process, suggests strategies for management, depending on which underlying pathology is suspected and identifies areas for future research for this common clinical presentation.

References

1. Finley CR, Chan DS, Garrison S, Korownyk C, Kolber MR, Campbell S, et al. What are the most common conditions in primary care? Systematic review. Can Fam Physician. 2018;64(11):832-40.
2. André M, Odenholt I, Schwan A, Axelsson I, Eriksson M, Hoffman M, et al. Upper respiratory tract infections in general practice: diagnosis, antibiotic prescribing, duration of symptoms and use of diagnostic tests. Scand J Infect Dis. 2002;34(12):880-6.
3. Komaroff AL, Pass TM, Aronson MD, Ervin CT, Cretin S, Winickoff RN, et al. The prediction of streptococcal pharyngitis in adults. J Gen Intern Med. 1986;1.
4. Hoffmann S. An Algorithm for a Selective use of Throat Swabs in the Diagnosis of Group: A Streptococcal Pharyngo-Tonsillitis in General Practice. Scandinavian Journal of Primary Health Care. 1992;10(4):295-300.
5. Komaroff AL, Aronson MD, Pass TM, Ervin CT, Branch WT, Jr., Schachter J. Serologic evidence of chlamydial and mycoplasmal pharyngitis in adults. Science. 1983;222(4626):927-9.
6. Del Mar C, Pincus D. Incidence patterns of respiratory illness in Queensland estimated from sentinel general practice. Australian family physician. 1995;24(4):625-9, 32.
7. Walsh BT, Bookheim WW, Johnson RC, Tompkins RK. Recognition of streptococcal pharyngitis in adults. Arch Intern Med. 1975;135.
8. Vincent MT, Celestin N, Hussain AN. Pharyngitis. Am Fam Physician. 2004;69(6):1465-70.
9. Ebell MH, Smith MA, Barry HC, Ives K, Carey M. The rational clinical examination. Does this patient have strep throat? Jama. 2000;284.
10. Hurt C, Tammaro D. Diagnostic evaluation of mononucleosis-like illnesses. The American journal of medicine. 2007;120(10):911 e1-8.
11. Grose C. The many faces of infectious mononucleosis: the spectrum of Epstein-Barr virus infection in children. Pediatrics in Review. 1985;7(2):35-44.
12. de-Thé G, Day NE, Geser A, Lavoué MF, Ho JH, Simons MJ, et al. Sero-epidemiology of the Epstein-Barr virus: preliminary analysis of an international study - a review. IARC Sci Publ. 1975(11 Pt 2):3-16.
13. Auwaerter PG. INfectious mononucleosis in middle age. Jama. 1999;281(5):454-9.
14. Straus SE, Cohen JI, Tosato G, Meier J. Epstein-Barr Virus Infections: Biology, Pathogenesis, and Management. Annals of internal medicine. 1993;118(1):45-58.
15. Kuri A, Jacobs BM, Vickaryous N, Pakpoor J, Middeldorp J, Giovannoni G, et al. Epidemiology of Epstein-Barr virus infection and infectious mononucleosis in the United Kingdom. BMC Public Health. 2020;20(1):912.
16. Gares V, Panico L, Castagne R, Delpierre C, Kelly-Irving M. The role of the early social environment on Epstein Barr virus infection: a prospective observational design using the Millennium Cohort Study. Epidemiol Infect. 2017;145(16):3405-12.
17. Rostgaard K, Wohlfahrt J, Hjalgrim H. A genetic basis for infectious mononucleosis: evidence from a family study of hospitalized cases in Denmark. Clin Infect Dis. 2014;58(12):1684-9.

18. Grotto I, Mimouni D, Huerta M, Mimouni M, Cohen D, Robin G, et al. Clinical and laboratory presentation of EBV positive infectious mononucleosis in young adults. Epidemiol Infect. 2003;131(1):683-9.
19. Visser E, Milne D, Collacott I, McLernon D, Counsell C, Vickers M. The epidemiology of infectious mononucleosis in Northern Scotland: a decreasing incidence and winter peak. BMC Infect Dis. 2014;14:151.
20. Henke CE, Kurland LT, Elveback LR. Infectious mononucleosis in Rochester, Minnesota, 1950 through 1969. American journal of epidemiology. 1973;98(6):483-90.
21. Brodsky AL, Heath CW, Jr. Infectious mononucleosis: epidemiologic patterns at United States colleges and universities. American journal of epidemiology. 1972;96(2):87-93.
22. Chang RS, Rosen L, Kapikian AZ. Epstein-Barr virus infections in a nursery. American journal of epidemiology. 1981;113(1):22-9.
23. Balfour HH, Holman Carol J, Hokanson Kristin M, Lelonek Meghan M, Giesbrecht Jill E, White Dana R, et al. A Prospective Clinical Study of Epstein-Barr Virus and Host Interactions during Acute Infectious Mononucleosis. Journal of Infectious Diseases. 2005;192(9):1505-12.
24. Balfour HH, Jr., Odumade OA, Schmeling DO, Mullan BD, Ed JA, Knight JA, et al. Behavioral, virologic, and immunologic factors associated with acquisition and severity of primary Epstein-Barr virus infection in university students. The Journal of infectious diseases. 2013;207(1):80-8.
25. Lennon P, Crotty M, Fenton JE. Infectious mononucleosis. BMJ (Clinical research ed). 2015;350.
26. Macsween KF, Higgins CD, McAulay KA, Williams H, Harrison N, Swerdlow AJ, et al. Infectious mononucleosis in university students in the United kingdom: evaluation of the clinical features and consequences of the disease. Clin Infect Dis. 2010;50(5):699-706.
27. Rea TD, Russo JE, Katon W, Ashley RL, Buchwald DS. Prospective study of the natural history of infectious mononucleosis caused by Epstein-Barr virus. The Journal of the American Board of Family Practice / American Board of Family Practice. 2001;14(4):234-42.
28. Thorley-Lawson DA, Miyashita EM, Khan G. Epstein-Barr virus and the B cell: that's all it takes. Trends in microbiology. 1996;4(5):204-8.
29. Hurley EA, Agger S, McNeil JA, Lawrence JB, Calendar A, Lenoir G, et al. When Epstein-Barr virus persistently infects B-cell lines, it frequently integrates. Journal of virology. 1991;65(3):1245-54.
30. Matsuo T, Heller M, Petti L, O'Shiro E, Kieff E. Persistence of the entire Epstein-Barr virus genome integrated into human lymphocyte DNA. Science. 1984;226(4680):1322-5.
31. Carter RL PH. Infectious Mononucleosis. Oxford and Edinburgh: Blackwell Scientific Publication; 1969.
32. Buchwald DS, Rea TD, Katon WJ, Russo JE, Ashley RL. Acute infectious mononucleosis: characteristics of patients who report failure to recover. The American journal of medicine. 2000;109(7):531-7.
33. Hickie I, Davenport T, Wakefield D, Vollmer-Conna U, Cameron B, Vernon SD, et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. BMJ (Clinical research ed). 2006;333(7568):575.
34. Cameron B, Bharadwaj M, Burrows J, Fazou C, Wakefield D, Hickie I, et al. Prolonged Illness after Infectious Mononucleosis Is Associated with Altered Immunity but Not with Increased Viral Load. Journal of Infectious Diseases. 2006;193(5):664-71.

35. Odame J, Robinson J, Khodai-Booran N, Yeung S, Mazzulli T, Stephens D, et al. Correlates of illness severity in infectious mononucleosis. Can J Infect Dis Med Microbiol. 2014;25(5):277-80.
36. Katz BZ, Reuter C, Lupovitch Y, Gleason K, McClellan D, Cotler J, et al. A Validated Scale for Assessing the Severity of Acute Infectious Mononucleosis. J Pediatr. 2019;209:130-3.
37. Charles PG. Infectious mononucleosis. Australian family physician. 2003;32(10):785-8.
38. Hoagland RJ. Infectious mononucleosis. Primary care. 1975;2(2):295-307.
39. Lennon P, O'Neill J, Fenton J, O'Dwyer T. Challenging the use of the lymphocyte to white cell count ratio in the diagnosis of infectious mononucleosis by analysis of a large cohort of Monospot test results. Clinical Otolaryngology. 2010;35(5):397-401.
40. Cai X, Ebell MH, Haines L. Accuracy of Signs, Symptoms, and Hematologic Parameters for the Diagnosis of Infectious Mononucleosis: A Systematic Review and Meta-Analysis. Journal of the American Board of Family Medicine : JABFM. 2021;34(6):1141-56.
41. Dunmire SK, Verghese PS, Balfour HH, Jr. Primary Epstein-Barr virus infection. J Clin Virol. 2018;102:84-92.
42. Linderholm M, Boman J, Juto P, Linde A. Comparative evaluation of nine kits for rapid diagnosis of infectious mononucleosis and Epstein-Barr virus-specific serology. J Clin Microbiol. 1994;32(1):259-61.
43. Hess RD. Routine Epstein-Barr virus diagnostics from the laboratory perspective: still challenging after 35 years. Journal of clinical microbiology. 2004;42(8):3381-7.
44. Luzuriaga K, Sullivan JL. Infectious mononucleosis. New England Journal of Medicine. 2010;362(21):1993-2000.
45. Bruu A-L, Hjetland R, Holter E, Mortensen L, Natås O, Petterson W, et al. Evaluation of 12 Commercial Tests for Detection of Epstein-Barr Virus-Specific and Heterophile Antibodies. Clinical and Diagnostic Laboratory Immunology. 2000;7(3):451-6.
46. Evans AS. Infectious mononucleosis and related syndromes. The American journal of the medical sciences. 1978;276(3):325-39.
47. Bartlett A, Williams R, Hilton M. Splenic rupture in infectious mononucleosis: a systematic review of published case reports. Injury. 2016;47(3):531-8.
48. Ramagopalan SV, Hoang U, Seagroatt V, Handel A, Ebers GC, Giovannoni G, et al. Geography of hospital admissions for multiple sclerosis in England and comparison with the geography of hospital admissions for infectious mononucleosis: a descriptive study. J Neurol Neurosurg Psychiatry. 2011;82(6):682-7.
49. Tattevin P, Le Tulzo Y, Minjolle S, Person A, Chapplain JM, Arvieux C, et al. Increasing incidence of severe Epstein-Barr virus-related infectious mononucleosis: surveillance study. J Clin Microbiol. 2006;44(5):1873-4.
50. Morris MC, Edmunds WJ. The Changing Epidemiology of Infectious Mononucleosis? Journal of Infection. 2002;45(2):107-9.
51. Lennon P, Saunders J, Fenton JE. A longer stay for the kissing disease: epidemiology of bacterial tonsillitis and infectious mononucleosis over a 20-year period. J Laryngol Otol. 2013;127(2):187-91.
52. Torre D, Tambini R. Acyclovir for treatment of infectious mononucleosis: a meta-analysis. Scandinavian Journal of Infectious Diseases. 1999;31(6):543-7.

53. Rezk E, Nofal YH, Hamzeh A, Aboujaib MF, AlKheder MA, Al Hammad MF. Steroids for symptom control in infectious mononucleosis. Cochrane Database Syst Rev. 2015;11:CD004402.
54. Jenson HB. Acute complications of Epstein-Barr virus infectious mononucleosis. Current opinion in pediatrics. 2000;12(3):263-8.
55. Lennon P, O'Neill JP, Fenton JE. Effect of metronidazole versus standard care on length of stay of patients admitted with severe infectious mononucleosis: a randomized controlled trial. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2014;20(7):O450-2.
56. Davidson S, Kaplinsky C, Frand M, Rotem J. Treatment of infectious mononucleosis with metronidazole in the pediatric age group. Scand J Infect Dis. 1982;14(2):103-4.
57. Hedström SA. Treatment of anginose infectious mononucleosis with metronidazole. A controlled clinical and laboratory study. Scand J Infect Dis. 1980;12(4):265-9.
58. Hedström SA, Mårdh PA, Ripa T. Treatment of anginose infectious mononucleosis with metronidazole. Scand J Infect Dis. 1978;10(1):7-9.
59. Spelman DW, Newton-John HF. Metronidazole in the treatment of anginose infectious mononucleosis. Scandinavian Journal of Infectious Diseases. 1982;14(2):99-101.
60. Thompson DF, Ramos CL. Antibiotic-Induced Rash in Patients With Infectious Mononucleosis. Ann Pharmacother. 2017;51(2):154-62.
61. Buchwald DS, Rea TD, Katon WJ, Russo JE, Ashley RL. Acute infectious mononucleosis: characteristics of patients who report failure to recover. The American journal of medicine. 2000;109(7):531-7.
62. White PD, Thomas JM, Kangro HO, Bruce-Jones WD, Amess J, Crawford DH, et al. Predictions and associations of fatigue syndromes and mood disorders that occur after infectious mononucleosis. Lancet (London, England). 2001;358(9297):1946-54.
63. Candy B, Chalder T, Cleare AJ, Wessely S, White PD, Hotopf M. Recovery from infectious mononucleosis: a case for more than symptomatic therapy? A systematic review. Br J Gen Pract. 2002;52(483):844-51.
64. Whitehead L. The Measurement of Fatigue in Chronic Illness: A Systematic Review of Unidimensional and Multidimensional Fatigue Measures. Journal of Pain and Symptom Management. 2009;37(1):107-28.
65. Neuberger GB. Measures of fatigue: The Fatigue Questionnaire, Fatigue Severity Scale, Multidimensional Assessment of Fatigue Scale, and Short Form-36 Vitality (Energy/Fatigue) Subscale of the Short Form Health Survey. Arthritis Care & Research. 2003;49(S5):S175-S83.
66. Epstein MA, Barr YM. CULTIVATION IN VITRO OF HUMAN LYMPHOBLASTS FROM BURKITT'S MALIGNANT LYMPHOMA. The Lancet. 1964;283(7327):252-3.
67. zur Hausen H, Schulte-Holthausen H, Klein G, Henle W, Henle G, Clifford P, et al. EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. Nature. 1970;228(5276):1056-8.
68. Hjalgrim H, Askling J, Rostgaard K, Hamilton-Dutoit S, Frisch M, Zhang J-S, et al. Characteristics of Hodgkin's Lymphoma after Infectious Mononucleosis. New England Journal of Medicine. 2003;349(14):1324-32.
69. Shibata D, Tokunaga M, Uemura Y, Sato E, Tanaka S, Weiss LM. Association of Epstein-Barr virus with undifferentiated gastric carcinomas with intense lymphoid infiltration. Lymphoepithelioma-like carcinoma. Am J Pathol. 1991;139(3):469-74.

70. Nielsen TR, Rostgaard K, Nielsen NM, Koch-Henriksen N, Haahr S, Sørensen PS, et al. Multiple sclerosis after infectious mononucleosis. Arch Neurol. 2007;64(1):72-5.
71. Bjornevik K, Cortese M, Healy BC, Kuhle J, Mina MJ, Leng Y, et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. Science.0(0).
72. Robinson WH, Steinman L. Epstein-Barr virus and multiple sclerosis. Science.0(0):eabm7930.
73. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. Lancet Infect Dis. 2005;5(11):685-94.
74. Centor RM, Witherspoon JM, Dalton HP, Brody CE, Link K. The diagnosis of strep throat in adults in the emergency room. Med Decis Making. 1981;1.
75. Oliver J, Malliya Wadu E, Pierse N, Moreland NJ, Williamson DA, Baker MG. Group A Streptococcus pharyngitis and pharyngeal carriage: A meta-analysis. PLOS Neglected Tropical Diseases. 2018;12(3):e0006335.
76. Graham A, Fahey T. Evidence based case report. Sore throat: diagnostic and therapeutic dilemmas. BMJ (Clinical research ed). 1999;319.
77. Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan EL, Lee G, et al. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. Clin Infect Dis. 2012;55(10):1279-82.
78. Bisno AL. Acute pharyngitis. N Engl J Med. 2001;344.
79. Neuner JM, Hamel MB, Phillips RS, Bona K, Aronson MD. Diagnosis and management of adults with pharyngitis. A cost-effectiveness analysis. Annals of internal medicine. 2003;139.
80. Cooper RJ, Hoffman JR, Bartlett JG, Besser RE, Gonzales R, Hickner JM, et al. Principles of appropriate antibiotic use for acute pharyngitis in adults: background. Annals of internal medicine. 2001;134(6):509-17.
81. Shaikh N, Leonard E, Martin JM. Prevalence of Streptococcal Pharyngitis and Streptococcal Carriage in Children: A Meta-analysis. Pediatrics. 2010;126(3):e557-e64.
82. Cohen JF, Bertille N, Cohen R, Chalumeau M. Rapid antigen detection test for group A streptococcus in children with pharyngitis. Cochrane Database Syst Rev. 2016(7).
83. Lean WL, Arnup S, Danchin M, Steer AC. Rapid diagnostic tests for group A streptococcal pharyngitis: a meta-analysis. Pediatrics. 2014;134(4):771-81.
84. Mantzourani E, Evans A, Cannings-John R, Ahmed H, Hood K, Reid N, et al. Impact of a pilot NHS-funded sore throat test and treat service in community pharmacies on provision and quality of patient care. BMJ Open Quality. 2020;9(1):e000833.
85. Blyth CC, Robertson PW. Anti-streptococcal antibodies in the diagnosis of acute and post-streptococcal disease: streptokinase versus streptolysin O and deoxyribonuclease B. Pathology. 2006;38(2):152-6.
86. Smieszek T, Pouwels KB, Dolk FCK, Smith DRM, Hopkins S, Sharland M, et al. Potential for reducing inappropriate antibiotic prescribing in English primary care. J Antimicrob Chemother. 2018;73(suppl_2):ii36-ii43.
87. Spinks A, Glasziou PP, Del Mar CB. Antibiotics for treatment of sore throat in children and adults. Cochrane Database Syst Rev. 2021(12).
88. Little P, Gould C, Williamson I, Warner G, Gantley M, Kinmonth AL. Reattendance and complications in a randomised trial of prescribing strategies for sore throat: the medicalising effect of prescribing antibiotics. BMJ (Clinical research ed). 1997;315(7104):350-2.

89. Committee HEA. Antibiotic prescribing, Pharyngitis / Sore Throat / Tonsillitis. 2021.
90. Little P, Hobbs FDR, Moore M, Mant D, Williamson I, McNulty C, et al. Clinical score and rapid antigen detection test to guide antibiotic use for sore throats: randomised controlled trial of PRISM (primary care streptococcal management). BMJ : British Medical Journal. 2013;347:f5806.
91. Excellence NNIfHaC. NICE guideline [NG84]: Sore throat (acute): antimicrobial prescribing.
92. Network SSIG. Management of sore throat and indications for tonsillectomy
A national clinical guideline. 2010.
93. Pelucchi C, Grigoryan L, Galeone C, Esposito S, Huovinen P, Little P, et al. Guideline for the management of acute sore throat: ESCMID Sore Throat Guideline Group. Clinical Microbiology and Infection. 2012;18, Supplement 1:1-27.
94. Skoog Ståhlgren G, Tyrstrup M, Edlund C, Giske CG, Mölstad S, Norman C, et al. Penicillin V four times daily for five days versus three times daily for 10 days in patients with pharyngotonsillitis caused by group A streptococci: randomised controlled, open label, non-inferiority study. BMJ (Clinical research ed). 2019;367:l5337.
95. Altamimi S, Khalil A, Khalaiwi KA, Milner RA, Pusic MV, Al Othman MA. Short-term late-generation antibiotics versus longer term penicillin for acute streptococcal pharyngitis in children. Cochrane Database Syst Rev. 2012(8).
96. Matthys J, De Meyere M, van Driel ML, De Sutter A. Differences among international pharyngitis guidelines: not just academic. Annals of family medicine. 2007;5(5):436-43.
97. Gunnarsson R, Ebell MH, Wächtler H, Manchal N, Reid L, Malmberg S, et al. Association between guidelines and medical practitioners' perception of best management for patients attending with an apparently uncomplicated acute sore throat: a cross-sectional survey in five countries. BMJ Open. 2020;10(9):e037884.
98. Banerjee S, Ford C. Clinical Decision Rules and Strategies for the Diagnosis of Group A Streptococcal Infection: A Review of Clinical Utility and Guidelines. Canadian Agency for Drugs and Technologies in Health, Ottawa (ON); 2018.
99. Schünemann HJ, Fretheim A, Oxman AD. Improving the use of research evidence in guideline development: 1. Guidelines for guidelines. Health Research Policy and Systems. 2006;4(1):1-6.
100. Aertgeerts B, Agoritsas T, Siemieniuk RAC, Burgers J, Bekkering GE, Merglen A, et al. Corticosteroids for sore throat: a clinical practice guideline. BMJ (Clinical research ed). 2017;358:j4090.
101. Hayward GN, Hay AD, Moore MV, Jawad S, Williams N, Voysey M, et al. Effect of Oral Dexamethasone Without Immediate Antibiotics vs Placebo on Acute Sore Throat in Adults: A Randomized Clinical Trial. Jama. 2017;317(15):1535-43.
102. Sadeghirad B, Siemieniuk RAC, Brignardello-Petersen R, Papola D, Lytvyn L, Vandvik PO, et al. Corticosteroids for treatment of sore throat: systematic review and meta-analysis of randomised trials. BMJ (Clinical research ed). 2017;358:j3887.
103. Chretien JH, Esswein JG. How frequent is bacterial superinfection of the pharynx in infectious mononucleosis? Observations on incidence, recognition, and management with antibiotics. Clin Pediatr (Phila). 1976;15(5):424-7.
104. Jarvis WR. A study of beta hemolytic streptococcal pharyngitis in patients with infectious mononucleosis. Clin Pediatr (Phila). 1980;19(7):463-7.
105. Merriam SC, Keeling RP. Beta-hemolytic streptococcal pharyngitis: uncommon in infectious mononucleosis. South Med J. 1983;76(5):575-6.

106. Hoffmann S. The Throat Carrier Rate of Group a and Other Beta Hemolytic Streptococci Among Patients in General Practice. Acta Pathologica Microbiologica Scandinavica Series B: Microbiology. 1985;93B(1-6):347-51.
107. Strömberg A, Schwan A, Cars O. Throat Carrier Rates of Beta-hemolytic Streptococci among Healthy Adults and Children. Scandinavian Journal of Infectious Diseases. 1988;20(4):411-7.
108. Begovac J, Bobinac E, Benic B, Desnica B, Maretic T, Basnec A, et al. Asymptomatic pharyngeal carriage of beta-haemolytic streptococci and streptococcal pharyngitis among patients at an urban hospital in Croatia. European Journal of Epidemiology. 1993;9(4):405-10.
109. Llor C, Madurell J, Balagué-Corbella M, Gómez M, Cots JM. Impact on antibiotic prescription of rapid antigen detection testing in acute pharyngitis in adults: a randomised clinical trial. Br J Gen Pract. 2011;61(586):e244-e51.
110. Thornton HV, Turner KME, Harrison S, Hammond A, Hawcroft C, Hay AD. Assessing the potential of upper respiratory tract point-of-care testing: a systematic review of the prognostic significance of upper respiratory tract microbes. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2019;25(11):1339-46.
111. Khalid TY, Duncan LJ, Thornton HV, Lasseter G, Muir P, Toney ZA, et al. Novel multi-virus rapid respiratory microbiological point-of-care testing in primary care: a mixed-methods feasibility evaluation. Fam Pract. 2021;38(5):598-605.
112. Butler CC, Gillespie D, White P, Bates J, Lowe R, Thomas-Jones E, et al. C-Reactive Protein Testing to Guide Antibiotic Prescribing for COPD Exacerbations. New England Journal of Medicine. 2019;381(2):111-20.
113. Boere TM, van Buul LW, Hopstaken RM, van Tulder MW, Twisk JWMR, Verheij TJM, et al. Effect of C reactive protein point-of-care testing on antibiotic prescribing for lower respiratory tract infections in nursing home residents: cluster randomised controlled trial. BMJ (Clinical research ed). 2021;374:n2198.
114. Cals JW, Butler CC, Hopstaken RM, Hood K, Dinant G-J. Effect of point of care testing for C reactive protein and training in communication skills on antibiotic use in lower respiratory tract infections: cluster randomised trial. BMJ (Clinical research ed). 2009;338.
115. Little P, Stuart B, Francis N, Douglas E, Tonkin-Crine S, Anthierens S, et al. Effects of internet-based training on antibiotic prescribing rates for acute respiratory-tract infections: a multinational, cluster, randomised, factorial, controlled trial. Lancet (London, England). 2013;382(9899):1175-82.
116. Hay AD. Point-of-care tests to inform antibiotic prescribing. BMJ (Clinical research ed). 2021;374:n2253.
117. Le Bel J, Hausfater P, Chenevier-Gobeaux C, Blanc F-X, Benjoar M, Ficko C, et al. Diagnostic accuracy of C-reactive protein and procalcitonin in suspected community-acquired pneumonia adults visiting emergency department and having a systematic thoracic CT scan. Critical Care. 2015;19(1):1-12.
118. van Vugt SF, Broekhuizen BD, Lammens C, Zuithoff NP, de Jong PA, Coenen S, et al. Use of serum C reactive protein and procalcitonin concentrations in addition to symptoms and signs to predict pneumonia in patients presenting to primary care with acute cough: diagnostic study. BMJ (Clinical research ed). 2013;346.

119. Bayes T. LII. An essay towards solving a problem in the doctrine of chances. By the late Rev. Mr. Bayes, FRS communicated by Mr. Price, in a letter to John Canton, AMFR S. Philosophical transactions of the Royal Society of London. 1763(53):370-418.
120. Bossuyt PM, Irwig L, Craig J, Glasziou P. Comparative accuracy: assessing new tests against existing diagnostic pathways. BMJ (Clinical research ed). 2006;332(7549):1089-92.
121. McGinn TG, Guyatt GH, Wyer PC, Naylor CD, Stiell IG, Richardson WS. Users' guides to the medical literature: XXII: how to use articles about clinical decision rules. Evidence-Based Medicine Working Group. Jama. 2000;284(1):79-84.
122. Adams ST, Leveson SH. Clinical prediction rules. BMJ (Clinical research ed). 2012;344:d8312.
123. Falk G, Fahey T. Clinical prediction rules. BMJ (Clinical research ed). 2009;339:b2899.
124. Galvin R, Joyce D, Downey E, Boland F, Fahey T, Hill AK. Development and validation of a clinical prediction rule to identify suspected breast cancer: a prospective cohort study. BMC Cancer. 2014;14:743.
125. Grobman WA, Stamilio DM. Methods of clinical prediction. American journal of obstetrics and gynecology. 2006;194(3):888-94.
126. Liao L, Mark DB. Clinical prediction models: are we building better mousetraps? J Am Coll Cardiol. 2003;42(5):851-3.
127. Ebell MH, Locatelli I, Senn N. A novel approach to the determination of clinical decision thresholds. Evid Based Med. 2015;20(2):41-7.
128. Dale AP, Marchello C, Ebell MH. Clinical gestalt to diagnose pneumonia, sinusitis, and pharyngitis: a meta-analysis. Br J Gen Pract. 2019;69(684):e444-e53.
129. Van den Bruel A, Thompson M, Buntinx F, Mant D. Clinicians' gut feeling about serious infections in children: observational study. BMJ : British Medical Journal. 2012;345:e6144.
130. Elstein AS, Kagan N, Shulman LS, Jason H, Loupe MJ. Methods and theory in the study of medical inquiry. J Med Educ. 1972;47(2):85-92.
131. Norman G, Barraclough K, Dolovich L, Price D. Iterative diagnosis. BMJ (Clinical research ed). 2009;339:b3490.
132. Heneghan C, Glasziou P, Thompson M, Rose P, Balla J, Lasserson D, et al. Diagnostic strategies used in primary care. BMJ (Clinical research ed). 2009;338:b946.
133. Doust J. Using probabilistic reasoning. BMJ (Clinical research ed). 2009;339:b3823.
134. Whiting P, Toerien M, de Salis I, Sterne JAC, Dieppe P, Egger M, et al. A review identifies and classifies reasons for ordering diagnostic tests. Journal of clinical epidemiology. 2007;60(10):981-9.
135. Howie JG. Diagnosis--the Achilles heel? J R Coll Gen Pract. 1972;22(118):310-5.
136. Jones R, Barraclough K, Dowrick C. When no diagnostic label is applied. BMJ (Clinical research ed). 2010;340:c2683.
137. Grimshaw JM, Russell IT. Effect of clinical guidelines on medical practice: a systematic review of rigorous evaluations. Lancet (London, England). 1993;342(8883):1317-22.
138. Kelly JT. Role of clinical practice guidelines and clinical profiling in facilitating optimal laboratory use. Clinical Chemistry. 1995;41(8):1234-6.
139. Freeborn DK, Shye D, Mullooly JP, Eraker S, Romeo J. Primary care physicians' use of lumbar spine imaging tests. Journal of General Internal Medicine. 1997;12(10):619-25.
140. Pauker SG, Kassirer JP. The Threshold Approach to Clinical Decision Making. New England Journal of Medicine. 1980;302(20):1109-17.

141. Ebell MH, Rahmatullah I, Cai X, Bentivegna M, Hulme C, Thompson M, et al. A Systematic Review of Clinical Prediction Rules for the Diagnosis of Influenza. The Journal of the American Board of Family Medicine. 2021;34(6):1123-40.
142. Guyatt G, Rennie D. Users' guides to the medical literature : a manual for evidence-based clinical practice. 2nd ed. ed. London: McGraw-Hill Medical; 2008.
143. Ebell MH, Locatelli I, Mueller Y, Senn N, Morgan K. Diagnosis and treatment of community-acquired pneumonia in patients with acute cough: A quantitative study of decision thresholds in primary care. British journal of general practice. 2018;68(676):e765-e74.
144. De Paor M, O'Brien K, Fahey T, Smith SM. Antiviral agents for infectious mononucleosis (glandular fever). Cochrane Database Syst Rev. 2016(12).
145. Ebell MH. Epstein-Barr virus infectious mononucleosis. Am Fam Physician. 2004;70(7):1279-87.
146. Kawano Y, Iwata S, Kawada J, Gotoh K, Suzuki M, Torii Y, et al. Plasma viral microRNA profiles reveal potential biomarkers for chronic active Epstein-Barr virus infection. The Journal of infectious diseases. 2013;208(5):771-9.
147. van de Veerdonk FL, Wever PC, Hermans MH, Fijnheer R, Joosten LA, van der Meer JW, et al. IL-18 serum concentration is markedly elevated in acute EBV infection and can serve as a marker for disease severity. The Journal of infectious diseases. 2012;206(2):197-201.
148. Monem SA, O'Connor PF, O'Leary TG. Peritonsillar abscess and infectious mononucleosis: an association or a different presentation of the same condition. Irish medical journal. 1999;92(2):278-80.
149. Aldrete JS. Spontaneous rupture of the spleen in patients with infectious mononucleosis. Mayo Clinic proceedings. 1992;67(9):910-2.
150. Brichkov I, Cummings L, Fazylov R, Horovitz JH. Nonoperative management of spontaneous splenic rupture in infectious mononucleosis: the role for emerging diagnostic and treatment modalities. The American surgeon. 2006;72(5):401-4.
151. Chen J, Konstantinopoulos PA, Satyal S, Telonis J, Blair DC. Just another simple case of infectious mononucleosis? Lancet (London, England). 2003;361(9364):1182.
152. Devereaux CE, Bemiller T, Brann O. Ascites and severe hepatitis complicating Epstein-Barr infection. The American journal of gastroenterology. 1999;94(1):236-40.
153. Lei PS, Lowichik A, Allen W, Mauch TJ. Acute renal failure: unusual complication of Epstein-Barr virus-induced infectious mononucleosis. Clin Infect Dis. 2000;31(6):1519-24.
154. Balfour HH, Jr., Hokanson KM, Schacherer RM, Fietzer CM, Schmeling DO, Holman CJ, et al. A virologic pilot study of valacyclovir in infectious mononucleosis. Journal of Clinical Virology. 2007;39(1):16-21.
155. Kimberlin DW WR. Antiviral therapy of HSV-1 and -2. In: Arvin A, Campadelli-Fiume G, Mocarski E, et al., editors. Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge: Cambridge University Press; 2007.
156. Aciclovir: Summary of Product Characteristics [Internet]. N/A. 2016 [cited 02/03/17]. Available from: https://www.hpra.ie/img/uploaded/swedocuments/LicenseSPC_PPA1151-028-002_26012015152037.pdf.
157. Candy B, Chalder T, Cleare AJ, Wessely S, White PD, Hotopf M. Recovery from infectious mononucleosis: a case for more than symptomatic therapy? A systematic review. The British journal of general practice : the journal of the Royal College of General Practitioners. 2002;52(483):844-51.

158. Lefebvre C GJ, Briscoe S, Littlewood A, Marshall C, Metzendorf M-I, Noel-Storr A, Rader T, Shokraneh F, Thomas J, Wieland LS. . Chapter 4: Searching for and selecting studies. In: Higgins JPT TJ, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors), editor. Cochrane Handbook for Systematic Reviews of Interventions Version 62: The Cochrane Collaboration; 2021.
159. Higgins JPT TJ, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions. Version 6.2 ed: The Cochrane Collaboration; 2021 Updated February 2021.
160. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. BMC medical research methodology. 2005;5:13.
161. Nordic Cochrane Centre TCC. Review Manager (RevMan). 2014.
162. GRADEpro. Available from gradeproorg. 2020.
163. Andersson J, Skoldenberg B, Ernberg I, Britton S, Henle W, Andersson U. Acyclovir treatment in primary Epstein-Barr virus infection. A double-blind placebo-controlled study. Scand J Infect Dis Suppl. 1985;47:107-15.
164. Andersson J, Britton S, Ernberg I, Andersson U, Henle W, Skoldenberg B, et al. Effect of acyclovir on infectious mononucleosis: a double-blind, placebo-controlled study. Journal of Infectious Diseases. 1986;153(2):283-90.
165. Ernberg I, Andersson J. Acyclovir efficiently inhibits oropharyngeal excretion of Epstein-Barr virus in patients with acute infectious mononucleosis. J Gen Virol. 1986;67(Pt 10):2267-72.
166. ChiCtr. A randomized controlled study of antiviral therapy in children with infectious mononucleosis. https://trialsearchwhoint/Trial2aspx?TrialID=ChiCTR2100043741. 2021.
167. Irct20201201049554N. Effectiveness of Oral Acyclovir in Treatment of Infectious Mononucleosis in Children. https://trialsearchwhoint/Trial2aspx?TrialID=IRCT20201201049554N1. 2021.
168. Andersson J, Skoldenberg B, Henle W, Giesecke J, Ortqvist A, Julander I, et al. Acyclovir treatment in infectious mononucleosis: a clinical and virological study. Infection. 1987;15 Suppl 1:S14-20.
169. Balfour HH, Jr. Activity of Valomaciclovir in Infectious Mononucleosis Due to Primary Epstein-Barr Virus Infection (Mono6). Clinicaltrialsgov. 2007.
170. Pagano JS, Sixbey JW, Lin JC. Acyclovir and Epstein-Barr virus infection. Journal of Antimicrobial Chemotherapy. 1983;12 Suppl B:113-21.
171. Simon MW, Deeter RG, Shahan B. The Effect of Valacyclovir and Prednisolone in Reducing Symptoms of EBV Illness in Children: A Double-Blind, Placebo-Controlled Study. International Pediatrics [Internet]. 2003; 18(3):[164-9 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/792/CN-00631792/frame.html.
172. van der Horst C, Joncas J, Ahronheim G, Gustafson N, Stein G, Gurwith M, et al. Lack of effect of peroral acyclovir for the treatment of acute infectious mononucleosis. Journal of Infectious Diseases. 1991;164(4):788-92.
173. Lexchin J, Bero LA, Djulbegovic B, Clark O. Pharmaceutical industry sponsorship and research outcome and quality: systematic review. BMJ (Clinical research ed). 2003;326(7400):1167-70.
174. Dobson J, Whitley RJ, Pocock S, Monto AS. Oseltamivir treatment for influenza in adults: a meta-analysis of randomised controlled trials. Lancet (London, England). 2015;385(9979):1729-37.

175. Gagyor I, Madhok VB, Daly F, Somasundara D, Sullivan M, Gammie F, et al. Antiviral treatment for Bell's palsy (idiopathic facial paralysis). Cochrane Database Syst Rev. 2015(11).
176. Klassen TP, Hartling L. Acyclovir for treating varicella in otherwise healthy children and adolescents. Cochrane Database Syst Rev. 2005(4).
177. Ebell MH, Call M, Shinholser J, Gardner J. Does This Patient Have Infectious Mononucleosis?: The Rational Clinical Examination Systematic Review. Jama. 2016;315(14):1502-9.
178. Han K, Song K, Choi BW. How to Develop, Validate, and Compare Clinical Prediction Models Involving Radiological Parameters: Study Design and Statistical Methods. Korean Journal of Radiology. 2016;17(3):339-50.
179. Vergouwe Y, Steyerberg EW, Eijkemans MJ, Habbema JD. Substantial effective sample sizes were required for external validation studies of predictive logistic regression models. J Clin Epidemiol. 2005;58(5):475-83.
180. Little P, Hobbs FD, Moore M, Mant D, Williamson I, McNulty C, et al. PRImary care Streptococcal Management (PRISM) study: in vitro study, diagnostic cohorts and a pragmatic adaptive randomised controlled trial with nested qualitative study and cost-effectiveness study. Health Technol Assess. 2014;18(6):vii-xxv, 1-101.
181. Cowley LE, Farewell DM, Maguire S, Kemp AM. Methodological standards for the development and evaluation of clinical prediction rules: a review of the literature. Diagnostic and Prognostic Research. 2019;3(1):16.
182. Alba AC, Agoritsas T, Walsh M, Hanna S, Iorio A, Devereaux PJ, et al. Discrimination and Calibration of Clinical Prediction Models: Users' Guides to the Medical Literature. Jama. 2017;318(14):1377-84.
183. Pencina MJ, D'Agostino RB. Evaluating discrimination of risk prediction models: the C statistic. Jama. 2015;314(10):1063-4.
184. Moons KGM, Altman DG, Reitsma JB, Ioannidis JPA, Macaskill P, Steyerberg EW, et al. Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD): Explanation and ElaborationThe TRIPOD Statement: Explanation and Elaboration. Annals of internal medicine. 2015;162(1):W1-W73.
185. StataCorp. Stata Statistical Software: Release 16. 2019.
186. Keogh C, Wallace E, O'Brien KK, Galvin R, Smith SM, Lewis C, et al. Developing an international register of clinical prediction rules for use in primary care: a descriptive analysis. Annals of family medicine. 2014;12(4):359-66.
187. Fahey T, Van der Lei J. Producing and Using Clinical Prediction Rules. The Evidence Base of Clinical Diagnosis: Theory and methods of diagnostic research, Second Edition: Blackwell Publishing Ltd; 2009.
188. Willis BH. Spectrum bias—why clinicians need to be cautious when applying diagnostic test studies. Family Practice. 2008;25(5):390-6.
189. Reilly BM, Evans AT. Translating clinical research into clinical practice: impact of using prediction rules to make decisions. Annals of internal medicine. 2006;144(3):201-9.
190. Toll DB, Janssen KJ, Vergouwe Y, Moons KG. Validation, updating and impact of clinical prediction rules: a review. J Clin Epidemiol. 2008;61(11):1085-94.
191. McIsaac WJ, Goel V, To T, Low DE. The validity of a sore throat score in family practice. CMAJ. 2000;163.
192. Hedin K, Bieber L, Lindh M, Sundqvist M. The aetiology of pharyngotonsillitis in adolescents and adults - Fusobacterium necrophorum is commonly found. Clinical

microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2015;21(3):263.e1-7.
193. Gerber MA, Shulman ST. Rapid diagnosis of pharyngitis caused by group A streptococci. Clin Microbiol Rev. 2004;17.
194. Cohen JF, Pauchard JY, Hjelm N, Cohen R, Chalumeau M. Efficacy and safety of rapid tests to guide antibiotic prescriptions for sore throat. Cochrane Database Syst Rev. 2020(6).
195. Reinholdt KB, Rusan M, Hansen PR, Klug TE. Management of sore throat in Danish general practices. BMC family practice. 2019;20(1):1-7.
196. Centor RM, Atkinson TP, Ratliff AE, Xiao L, Crabb DM, Estrada CA, et al. The clinical presentation of Fusobacterium-positive and streptococcal-positive pharyngitis in a university health clinic: a cross-sectional study. Annals of internal medicine. 2015;162(4):241-7.
197. McIsaac WJ, White D, Tannenbaum D, Low DE. A clinical score to reduce unnecessary antibiotic use in patients with sore throat. Canadian Medical Association Journal. 1998;158(1):75-83.
198. Aalbers J, O'Brien KK, Chan W-S, Falk GA, Teljeur C, Dimitrov BD, et al. Predicting streptococcal pharyngitis in adults in primary care: a systematic review of the diagnostic accuracy of symptoms and signs and validation of the Centor score. BMC Medicine. 2011;9(1):1-11.
199. Willis BH, Coomar D, Baragilly M. Comparison of Centor and McIsaac scores in primary care: a meta-analysis over multiple thresholds. British Journal of General Practice. 2020;70(693):e245-e54.
200. Fine AM, Nizet V, Mandl KD. Large-scale validation of the Centor and McIsaac scores to predict group A streptococcal pharyngitis. Arch Intern Med. 2012;172(11):847-52.
201. Seeley A, Fanshawe T, Voysey M, Hay A, Moore M, Hayward G. Diagnostic accuracy of Fever-PAIN and Centor criteria for bacterial throat infection in adults with sore throat: a secondary analysis of a randomised controlled trial. BJGP Open. 2021;5(6).
202. McGinn TG, Deluca J, Ahlawat SK, Mobo BH, Jr., Wisnivesky JP. Validation and modification of streptococcal pharyngitis clinical prediction rules. Mayo Clinic proceedings. 2003;78(3):289-93.
203. Linder JA, Stafford RS. Antibiotic treatment of adults with sore throat by community primary care physicians: a national survey, 1989-1999. Jama. 2001;286.
204. Stuart B, Brotherwood H, Van't Hoff C, Brown A, van den Bruel A, Hay AD, et al. Exploring the appropriateness of antibiotic prescribing for common respiratory tract infections in UK primary care. Journal of Antimicrobial Chemotherapy. 2019;75(1):236-42.
205. Arnold SR, Straus SE. Interventions to improve antibiotic prescribing practices in ambulatory care. Cochrane Database Syst Rev. 2005(4).
206. Razai M, Hussain K. Improving antimicrobial prescribing practice for sore throat symptoms in a general practice setting. BMJ Qual Improv Rep. 2017;6(1):u211706.w4738.
207. Lambore S, McSherry J, Kraus AS. Acute and chronic symptoms of mononucleosis. J Fam Pract. 1991;33(1):33-7.
208. Katz BZ, Shiraishi Y, Mears CJ, Binns HJ, Taylor R. Chronic Fatigue Syndrome Following Infectious Mononucleosis in Adolescents: A Prospective Cohort Study. Pediatrics. 2009;124(1):189-93.
209. White PD, Thomas JM, Amess J, Crawford DH, Grover SA, Kangro HO, et al. Incidence, risk and prognosis of acute and chronic fatigue syndromes and psychiatric disorders after glandular fever. The British Journal of Psychiatry. 1998;173(6):475-81.

210. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Annals of internal medicine. 1994;121(12):953-9.
211. Kerr JR, Bracewell J, Laing I, Mattey DL, Bernstein RM, Bruce IN, et al. Chronic fatigue syndrome and arthralgia following parvovirus B19 infection. The Journal of Rheumatology. 2002;29(3):595-602.
212. Pawlikowska T, Chalder T, Hirsch SR, Wallace P, Wright DJM, Wessely SC. Population based study of fatigue and psychological distress. BMJ (Clinical research ed). 1994;308(6931):763-6.
213. Vollmer-Conna U, Aslakson E, White PD. An empirical delineation of the heterogeneity of chronic unexplained fatigue in women. Pharmacogenomics. 2006;7(3):355-64.
214. Jason LA, Katz BZ, Shiraishi Y, Mears CJ, Im Y, Taylor RR. Predictors of post-infectious chronic fatigue syndrome in adolescents. Health Psychology and Behavioral Medicine. 2014;2(1):41-51.
215. Pedersen M, Asprusten TT, Godang K, Leegaard TM, Osnes LT, Skovlund E, et al. Predictors of chronic fatigue in adolescents six months after acute Epstein-Barr virus infection: A prospective cohort study. Brain Behav Immun. 2019;75:94-100.
216. Petersen I, Thomas JM, Hamilton WT, White PD. Risk and predictors of fatigue after infectious mononucleosis in a large primary-care cohort. QJM: An International Journal of Medicine. 2005;99(1):49-55.
217. Cameron B, Galbraith S, Zhang Y, Davenport T, Vollmer-Conna U, Wakefield D, et al. Gene Expression Correlates of Postinfective Fatigue Syndrome after Infectious Mononucleosis. The Journal of infectious diseases. 2007;196(1):56-66.
218. Candy B, Chalder T, Cleare AJ, Peakman A, Skowera A, Wessely S, et al. Predictors of fatigue following the onset of infectious mononucleosis. Psychological medicine. 2003;33(5):847-55.
219. Greenfield NS, Roessler R, Crosley AP, Jr. Ego strength and length of recovery from infectious mononucleosis. J Nerv Ment Dis. 1959;128(2):125-8.
220. Dalrymple W. INFECTIOUS MONONUCLEOSIS. 2. RELATION OF BED REST AND ACTIVITY TO PROGNOSIS. Postgrad Med. 1964;35:345-9.
221. Thompson DS, Godleski J, Herman S. Prognosis post infectious mononucleosis. J Am Coll Health Assoc. 1969;17(5):453-7.
222. Cadie M, Nye FJ, Storey P. Anxiety and Depression After Infectious Mononucleosis. British Journal of Psychiatry. 1976;128(6):559-61.
223. Chretien JH, Esswein JG, Holland WG, McCauley CE. Predictors of the duration of infectious mononucleosis. South Med J. 1977;70(4):437-9.
224. Katon W, Russo J, Ashley RL, Buchwald D. Infectious mononucleosis: psychological symptoms during acute and subacute phases of illness. General hospital psychiatry. 1999;21(1):21-9.
225. Dittner AJ, Wessely SC, Brown RG. The assessment of fatigue: A practical guide for clinicians and researchers. Journal of Psychosomatic Research. 2004;56(2):157-70.
226. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol. 1989;46(10):1121-3.

227. Armutlu K, Korkmaz NC, Keser I, Sumbuloglu V, Akbiyik DI, Guney Z, et al. The validity and reliability of the Fatigue Severity Scale in Turkish multiple sclerosis patients. Int J Rehabil Res. 2007;30(1):81-5.
228. Valko PO, Bassetti CL, Bloch KE, Held U, Baumann CR. Validation of the fatigue severity scale in a Swiss cohort. Sleep. 2008;31(11):1601-7.
229. Rosti-Otajärvi E, Hämäläinen P, Wiksten A, Hakkarainen T, Ruutiainen J. Validity and reliability of the Fatigue Severity Scale in Finnish multiple sclerosis patients. Brain Behav. 2017;7(7):e00743.
230. Heller GZ, Manuguerra M, Chow R. How to analyze the Visual Analogue Scale: Myths, truths and clinical relevance. Scand J Pain. 2016;13:67-75.
231. Candy B, Chalder T, Cleare AJ, Wessely S, Hotopf M. A randomised controlled trial of a psycho-educational intervention to aid recovery in infectious mononucleosis. J Psychosom Res. 2004;57(1):89-94.
232. Powell P, Bentall RP, Nye FJ, Edwards RH. Randomised controlled trial of patient education to encourage graded exercise in chronic fatigue syndrome. BMJ (Clinical research ed). 2001;322(7283):387-90.
233. Prins JB, Bleijenberg G, Bazelmans E, Elving LD, de Boo TM, Severens JL, et al. Cognitive behaviour therapy for chronic fatigue syndrome: a multicentre randomised controlled trial. Lancet (London, England). 2001;357(9259):841-7.
234. Chalder T, Wallace P, Wessely S. Self-help treatment of chronic fatigue in the community: A randomized controlled trial. British Journal of Health Psychology. 1997;2(Part 3):189-97.
235. Ordóñez-Mena JM, Fanshawe TR, Butler CC, Mant D, Longhurst D, Muir P, et al. Relationship between microbiology of throat swab and clinical course among primary care patients with acute cough: a prospective cohort study. Fam Pract. 2020;37(3):332-9.
236. Verheij T, Cianci D, van der Velden A, Butler CC, Bongard E, Coenen S, et al. Clinical presentation and disease course in patients with flu-like illness: does microbiological aetiology matter? Br J Gen Pract. 2021.
237. Nalbandian A, Sehgal K, Gupta A, Madhavan MV, McGroder C, Stevens JS, et al. Post-acute COVID-19 syndrome. Nature Medicine. 2021;27(4):601-15.
238. Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD): The TRIPOD Statement. Annals of internal medicine. 2015;162(1):55-63.
239. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol. 2008;61(4):344-9.
240. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6.
241. Butler CC, van der Velden AW, Bongard E, Saville BR, Holmes J, Coenen S, et al. Oseltamivir plus usual care versus usual care for influenza-like illness in primary care: an open-label, pragmatic, randomised controlled trial. The Lancet (British edition). 2020;395(10217):42-52.
242. Matheson BA, Chisholm SM, Ho-Yen DO. Assessment of rapid ELISA test for detection of Epstein-Barr virus infection. J Clin Pathol. 1990;43(8):691-3.
243. Bhavnani M, McGucken RB, Craske J. Rapid ELISA for detecting Epstein-Barr virus infection. J Clin Pathol. 1991;44(4):351.

244. Tyrstrup M, van der Velden A, Engstrom S, Goderis G, Molstad S, Verheij T, et al. Antibiotic prescribing in relation to diagnoses and consultation rates in Belgium, the Netherlands and Sweden: use of European quality indicators. Scandinavian Journal of Primary Health Care. 2017;35(1):10-8.
245. Barnett ML, Ray KN, Souza J, Mehrotra A. Trends in Telemedicine Use in a Large Commercially Insured Population, 2005-2017. Jama. 2018;320(20):2147-9.
246. Martinez KA, Rood M, Jhangiani N, Kou L, Boissy A, Rothberg MB. Association Between Antibiotic Prescribing for Respiratory Tract Infections and Patient Satisfaction in Direct-to-Consumer Telemedicine. JAMA Internal Medicine. 2018;178(11):1558-60.

Appendices

Appendix 1: STROBE checklist for prospective cohort studies

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Section 3.2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	'Summary' section
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Chapter 1
Objectives	3	State specific objectives, including any prespecified hypotheses	Section 1.8
Study design	4	Present key elements of study design early in the paper	Chapter 3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Sections 3.2.1, 3.2.2, 3.2.3, 3.2.4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Sections 3.2.1, 3.2.2, 3.2.3, 3.4

			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed	N/A
			Case-control study—For matched studies, give matching criteria and the number of controls per case	N/A
Variables		7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Sections 3.2.4, 3.2.5
Data sources/ measurement		8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Sections 3.2.3, 3.2.4, 3.2.5, 7.3
Bias		9	Describe any efforts to address potential sources of bias	Section 3.3
Study size		10	Explain how the study size was arrived at	Section 3.2.6
Quantitative variables	11		Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Section 3.2.4
Statistical methods	12		(*a*) Describe all statistical methods, including those used to control for confounding	Section 7.3.4
			(*b*) Describe any methods used to examine subgroups and interactions	N/A
			(*c*) Explain how missing data were addressed	Section 7.3.4
			(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	Section 3.2.3
			Case-control study—If applicable, explain how matching of cases and controls was addressed	N/A
			Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	N/A
			(*e*) Describe any sensitivity analyses	N/A
Results				
Participants	13*		(a) Report numbers of individuals at each stage of study—e.g., numbers potentially eligible,	Section 3.4.1, 7.4

		examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	Section 3.4.1, 7.4
		(c) Consider use of a flow diagram	Section 7.4
Descriptive data	14*	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders	Section 3.4.1
		(b) Indicate number of participants with missing data for each variable of interest	Section 3.4.4
		(c) *Cohort study*—Summarise follow-up time (e.g., average and total amount)	Section 3.2.3, 7.4
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Section 3.4.3, 3.4.1, 7.4
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A
		Cross-sectional study—Report numbers of outcome events or summary measures	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Section 7.4
		(*b*) Report category boundaries when continuous variables were categorized	Section 7.4.1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	Section 7.5.1

Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Section 7.5.5
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Section 7.5
Generalisability	21	Discuss the generalisability (external validity) of the study results	Section 7.5.3
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Acknowledgments page

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Appendix 2: TRIPOD checklist: prediction model development

Section/Topic		Checklist Item	Section
Title and abstract			
Title		Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	Chapter 4
Abstract		Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	N/A
Introduction			
Background and objectives		Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	4.1
		Specify the objectives, including whether the study describes the development or validation of the model or both.	4.2
Methods			
Source of data		Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	Chapter 2
		Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	Chapter 2
Participants		Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	Chapter 2
		Describe eligibility criteria for participants.	Chapter 2
		Give details of treatments received, if relevant.	N/A
Outcome		Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	4.3.1
		Report any actions to blind assessment of the outcome to be predicted.	3.3
Predictors		Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	3.2.6
		Report any actions to blind assessment of predictors for the outcome and other predictors.	3.3
Sample size		Explain how the study size was arrived at.	3.2.6
Missing data		Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	4.3.4
Statistical analysis methods		Describe how predictors were handled in the analyses.	4.3.4
		Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	4.3.4
		Specify all measures used to assess model performance and, if relevant, to compare multiple models.	4.3.4
Risk groups		Provide details on how risk groups were created, if done.	N/A
Results			

Participants		Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	4.4.1
		Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	4.4.1
Model development		Specify the number of participants and outcome events in each analysis.	4.4.1
		If done, report the unadjusted association between each candidate predictor and outcome.	Table 4-1
Model specification		Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	Table 4-2
		Explain how to the use the prediction model.	4.5.1
Model performance		Report performance measures (with CIs) for the prediction model.	Table 4-4
Discussion			
Limitations		Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	4.5.6
Interpretation		Give an overall interpretation of the results, considering objectives, limitations, and results from similar studies, and other relevant evidence.	4.5
Implications		Discuss the potential clinical use of the model and implications for future research.	4.5.4
Other information			
Supplementary information		Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	Appendices
Funding		Give the source of funding and the role of the funders for the present study.	Acknowledgments page

Appendix 3: TRIPOD checklist: prediction model validation (EBV CPR score)

Section/Topic	ı	Checklist Item	Section
Title and abstract			
Title		Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	Chapter 5
Abstract		Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	N/A
Introduction			
Background and objectives		Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	5.1
		Specify the objectives, including whether the study describes the development or validation of the model or both.	5.2
Methods			
Source of data		Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	5.3.1
		Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	-
Participants		Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	5.3.1
		Describe eligibility criteria for participants.	5.3.1
		Give details of treatments received, if relevant.	N/A
Outcome		Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	5.3.2
		Report any actions to blind assessment of the outcome to be predicted.	N/A
Predictors		Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	5.3.4
		Report any actions to blind assessment of predictors for the outcome and other predictors.	N/A
Sample size		Explain how the study size was arrived at.	5.3.3
Missing data		Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	5.3.5
Statistical analysis methods		For validation, describe how the predictions were calculated.	5.3.5
		Specify all measures used to assess model performance and, if relevant, to compare multiple models.	5.3.5
		Describe any model updating (e.g., recalibration) arising from the validation, if done.	N/A
Risk groups		Provide details on how risk groups were created, if done.	N/A
Development vs. validation		For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	5.3.6

Results			
Participants		Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	5.4
		Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	5.4
		For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	Table 5-1
Model performance		Report performance measures (with CIs) for the prediction model.	Table 5-3
Model-updating		If done, report the results from any model updating (i.e., model specification, model performance).	N/A
Discussion			
Limitations		Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	5.5.3
Interpretation		For validation, discuss the results with reference to performance in the development data, and any other validation data.	5.5.2
		Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	5.5
Implications		Discuss the potential clinical use of the model and implications for future research.	5.5.4, 5.5.5
Other information			
Supplementary information		Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	Appendices
Funding		Give the source of funding and the role of the funders for the present study.	Acknowledgements page

Appendix 4: TRIPOD checklist: prediction model validation (Centor score)

Section/Topic	ı	Checklist Item	Section
Title and abstract			
Title		Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	Chapter 6
Abstract		Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	N/A
Introduction			
Background and objectives		Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	6.1
		Specify the objectives, including whether the study describes the development or validation of the model or both.	6.2
Methods			
Source of data		Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	Chapter 2
		Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	Chapter 2
Participants		Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	Chapter 2
		Describe eligibility criteria for participants.	Chapter 2
		Give details of treatments received, if relevant.	N/A
Outcome		Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	6.3.2
		Report any actions to blind assessment of the outcome to be predicted.	N/A
Predictors		Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	3.2.3, 6.3.2.1
		Report any actions to blind assessment of predictors for the outcome and other predictors.	N/A
Sample size		Explain how the study size was arrived at.	6.3.5
Missing data		Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	6.3.5
Statistical analysis methods		For validation, describe how the predictions were calculated.	6.3.5
		Specify all measures used to assess model performance and, if relevant, to compare multiple models.	6.3.5
		Describe any model updating (e.g., recalibration) arising from the validation, if done.	6.3.5
Risk groups		Provide details on how risk groups were created, if done.	N/A

Development vs. validation		For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	6.3.4.1
Results			
Participants		Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	6.4
		Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	6.4, 3.4
		For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	6.3.4.1
Model performance		Report performance measures (with CIs) for the prediction model.	Table 6-4
Model-updating		If done, report the results from any model updating (i.e., model specification, model performance).	6.4.2, 6.4.3
Discussion			
Limitations		Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	6.5.3
Interpretation		For validation, discuss the results with reference to performance in the development data, and any other validation data.	6.5.2
		Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	6.5
Implications		Discuss the potential clinical use of the model and implications for future research.	6.5.4, 6.5.5
Other information			
Supplementary information		Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	Appendices
Funding		Give the source of funding and the role of the funders for the present study.	Acknowledgements page

Appendix 5: Ethical approval and amendments for cohort study

Royal College of Surgeons in Ireland
The Research Ethics Committee
121 St. Stephens Green, Dublin 2, Ireland.
Tel: +353 1 4022205 Email: recadmin@rcsi.ie

RCSI
ROYAL COLLEGE OF SURGEONS IN IRELAND
COLÁISTE RÍOGA NA MÁINLEÁ IN ÉIRINN

Dr David Smith, Acting Chair
Dr Niamh Clarke, Convenor

2nd March 2017

Dr Muireann de Paor
Dept of General Practice,
Beaux Lane House,
Mercer St Lower,
Dublin 2.

Ethics Reference No:	REC 1352
Project Title:	An Irish primary care prospective cohort study; to derive and validate a clinical prediction rule to improve the diagnosis of Infectious Mononucleosis caused by Epstein Barr Virus; to examine the natural history of Infectious Mononucleosis caused by Epstein Barr Virus; and to validate the Centor criteria for Group A Streptococcal Pharyngitis.
Researchers Name (lead applicant):	Dr Muireann de Paor (RCSI Department of General Practice)
Principal investigator on the project (PI):	Prof Tom Fahy (RCSI Department of General Practice)
Other Individuals Involved:	Prof Susan Smith (RCSI Department of General Practice) and Dr Alan O' Donoghue (Mercers Medical Centre, Stephen Street Lower, D2)

Dear Dr de Paor,
Thank you for your Research Ethics Committee (REC) application. We are pleased to advise that ethical approval has been granted by the committee for this study.

This letter provides approval for data collection for the time requested in your application and for an additional 6 months. This is to allow for any unexpected delays in proceeding with data collection. Therefore this research ethics approval will expire on **15th October 2017.**

Where data collection is necessary beyond this point, approval for an extension must be sought from the Research Ethics Committee.

This ethical approval is given on the understanding that:

- All personnel listed in the approved application have read, understand and are thoroughly familiar with all aspects of the study.
- Any significant change which occurs in connection with this study and/or which may alter its ethical consideration must be reported immediately to the REC, and an ethical amendment submitted where appropriate.
- **A final report will be submitted to the REC upon completion of the project.**

We wish you all the best with your research.

Yours sincerely,

Niamh Clarke

PP Dr Niamh Clarke (Convenor)
Dr David Smith (Acting Chair)

Royal College of Surgeons in Ireland
The Research Ethics Committee
121 St. Stephens Green, Dublin 2, Ireland.
Tel: +353 1 4022205 Email: recadmin@rcsi.ie

RCSI
ROYAL COLLEGE OF SURGEONS IN IRELAND
COLÁISTE RÍOGA NA MÁINLEÁ IN ÉIRINN

15th May 2017

Dr Muireann de Paor
RCSI Department of General Practice
Beaux Lane House,
Mercer St Lower,
Dublin 2

Ethics Reference No:	REC1352b
Project Title:	An Irish primary care prospective cohort study; to derive and validate a clinical prediction rule to improve the diagnosis of
Researchers Name (lead applicant):	Dr Muireann de Paor (RCSI Department of General Practice)
Principal investigator on the project	Prof Tom Fahy (RCSI Department of General Practice)
Other Individuals Involved:	Prof Susan Smith (RCSI Department of General Practice) and Dr Alan O' Donoghue (Mercers Medical Centre, Stephen Street Lower,

Dear Dr de Paor,
Thank you for your amendment to Research Ethics Committee (REC) application REC1352. We are pleased to advise that ethical approval has been granted by the committee for this amendment.

Content of Amendment:

- Change to PIL and inclusion of a poster to reflect the draw for participants.
- Time extension request of 18 months
- Based on the pilot study 20 participants per site (15-20 sites) are expected.

This letter provides approval for data collection for the time requested in your original application and for an additional 6 months to allow for any unexpected delays in proceeding with data collection. Therefore this research ethics approval will expire on **15th October 2019**. Where data collection is necessary beyond this point, approval for an extension must be sought from the Research Ethics Committee.

This ethical approval is given on the understanding that:

- All personnel listed in the approved application have read, understand and are thoroughly familiar with all aspects of the study.
- Any significant change which occurs in connection with this study and/or which may alter its ethical consideration, must be reported immediately to the REC, and an ethical amendment submitted where appropriate.
- **A final report will be submitted to the REC upon completion of the project.**

We wish you all the best with your research.

Yours sincerely,

Niamh Clarke

PP Dr Niamh Clarke (Convenor)
Dr David Smith (Acting Chair)

Royal College of Surgeons in Ireland
The Research Ethics Committee
111 St. Stephens Green, Dublin 2, Ireland.
Tel: +353 1 4022205 Email: recadmin@rcsi.ie

Dr David Smith, Acting Chair
Dr Niamh Clarke, Convenor

08 AUGUST 2019
Prof Tom Fahey
RCSI Dept of General Practice

REC Number	REC201908006
Project Title	(Amendment to REC1352bbb) "An Irish primary care prospective cohort study; to derive and validate a clinical prediction rule to improve the diagnosis of Infectious Mononucleosis caused by Epstein Barr Virus
Principal Investigator:	Tom Fahey (RCSI Dept of General Practice)
Other Individuals Involved:	Muireann de Paor (RCSI Dept of General Practice)

Dear Prof Fahey
Thank you for your amendment to Research Ethics Committee (REC) application REC001352bbb. We are pleased to advise that ethical approval has been granted by the committee for this amendment.

Content of Amendment: Time extension until end of 2021 and increase to number of participants to 500

This letter provides approval for data collection for the time requested in your original application and for an additional 6 months to allow for any unexpected delays in proceeding with data collection. Therefore this research ethics approval will expire on 15 DECEMBER 2021. Where data collection is necessary beyond this point, approval for an extension must be sought from the REC.

This ethical approval is given on the understanding that:

- All personnel listed in the approved application have read, understand and are thoroughly familiar with all aspects of the study.
- Any significant change which occurs in connection with this study and/or which may alter its ethical consideration must be reported to the REC, and an ethical amendment submitted where appropriate.
- **A final report will be submitted to the REC upon completion of the project.**

This submission has been reviewed from an ethical perspective only. It is the responsibility of the PI/sponsor/data controller and relevant DPO to ensure and monitor compliance with relevant legislation in the country where the study is due to take place or any local policy in the site where the study is due to take place.

We wish you all the best with your research.
Yours sincerely,

Niamh Clarke

PP Dr Niamh Clarke (Convenor)
Dr David Smith (Acting Chair)

Appendix 6: Participant Information Sheet

HRB CENTRE FOR PRIMARY CARE RESEARCH DEPARTMENT OF GENERAL PRACTICE

Division of Population Health Sciences Royal College of Surgeons in Ireland

Beaux Lane House, Mercer Street Lower, Dublin 2, Ireland

Tel +353 1 4022473 **Fax** +353 1 4022764

Email enquiries.cpcr@rcsi.ie **www**.hrbcentreprimarycare.ie

A study of Infectious Mononucleosis (Glandular Fever) in Primary Care

PARTICIPANT INFORMATION SHEET (LONGER VERSION)

You are being invited to take part in a research study. Before you decide if you would like to take part it is important that you understand why we are doing this research and what it would involve for you. Please take time to read the following information carefully and decide whether or not you wish to take part. You may like to talk to others, friends or family members, about the study. Please ask if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

We want to find out more about infectious mononucleosis (glandular fever) which is a viral infection that causes sore throat, swollen lymph glands and sometimes other symptoms. We want to know what proportions of people with symptoms of glandular fever actually really have the condition when we do blood tests to check. We also want to find out how many people who test positive for glandular fever will end up missing time from college or usual activities or will end up having symptoms over a longer period.

In addition, we will be examining which symptoms best predict a diagnosis of real actual glandular fever. We will be developing a prediction rule in which researchers try to identify

the best combination of medical signs and symptoms in predicting the probability of glandular fever.

The final aim of our study is to test an established prediction rule for a bacterial cause of sore throat and see how that performs in an Irish setting.

Why have I been invited to take part?

We are inviting people to take part who are visiting or have telephoned their doctor to seek medical advice for glandular fever type symptoms. Your doctor will assess whether you are eligible for the study.

Do I have to take part?

No. It is your decision whether to be part of this study. Even if you decide to take part you are free to withdraw at any time without giving a reason, and this would not affect the standard of care you receive in any way. If you wish to withdraw from the study, please contact the trial team using the contact details at the bottom of this leaflet.

What happens in the study?

You will first be seen by your doctor or nurse in the usual way to assess your illness. As part of this visit the doctor or nurse will tell you about the trial and will check to see if you are eligible to take part. They will do this by:

- Going through a check list of symptoms and recording their severity.
- Checking the medical conditions you have and some basic details about you.

You will then be given time to think about whether you would like to take part in the trial, you will be able to ring friends or family members if you wish to discuss taking part with them.

If you are interested in taking part you will see a doctor or nurse who will:

- Give you the opportunity to ask any questions you may have.
- Ask you to sign a written consent form to say you are voluntarily taking part in the trial and you understand what is involved.
- Go through recruitment procedures. This will include recording your symptoms in more detail, examining you and taking a throat swab and a blood test.

- Complete forms about your symptoms, and explain to you about the follow up details that will be required at 3 and 6 months after the initial visit.

This must all happen within 7 days of your symptoms starting.

At 3 and 6 months after your initial visit you will be expected to complete a short online questionnaire or receive a phone call from the study team about whether your symptoms are resolving and if you have had any complications from the glandular fever. Completing this follow up information will take approximately 5 minutes at each time point. After this your participation in the study is complete.

You will be entered into a prize draw for a 250 euro One4all gift voucher.

Will I be required to take any study medication?

No. This study is not testing any medication. Your GP may advise over the counter medication or might possibly prescribe other medication but this would occur in the normal course of the consultation and is not part of the study process.

Will my taking part in the study be kept confidential?

Yes. If you join the study, some parts of your medical records may be looked at by authorised members of the research team and people checking that the study is being carried out correctly. The information collected for the study will be stored securely with no personal identifiers attached (such as name or address) and to which only authorised personnel will have access. We will be collecting your personal contact details to gather some follow-up information for the trial, but as soon as the information has been gathered your contact details will be anonymised.

What are the possible disadvantages or side effects of taking part?

Your consultation will likely take a few minutes longer than usual. You will be required to have a blood test and a throat swab that your GP may or may not have done during the usual course of the consultation if you weren't taking part in the study. We are asking you to complete two short online questionnaires for the research team.

What are the possible benefits of taking part?

You will have a throat swab and blood tests that you may not have had done as part of routine management. These may help you to get an earlier diagnosis. You will also be helping research to improve the diagnosis and management of glandular fever in the future.

Expenses and Payments

You will not be paid for taking part in this research study but you will be entered into a prize draw for a 250 euro One4all gift voucher.

Who is organising and funding the research?

The research has been organised by GP researchers in the HRB Centre for Primary Care Research in the Royal College of Surgeons in Ireland (RCSI).

What happens if I don't want to carry on with the study?

You can withdraw from the study at any time without giving a reason. Withdrawing from the study will not affect your future medical care. If you wish to withdraw from the study, please contact the trial team using the contact details on the bottom of this leaflet. The research team will still use the data collected up to your withdrawal unless you tell us at the time that you withdraw that you would prefer us not to.

What will happen to any samples I give?

We are asking you to have a throat swab and a blood sample taken on the day you join the trial. We will send these samples to the local laboratory for testing to see whether you have glandular fever or whether you might have a bacterial cause of sore throat. We will use this information to help us understand which symptoms and medical signs are the best predictors of a bacterial cause of sore throat or glandular fever, and to examine what type of complications and symptoms are associated with glandular fever.

What will happen to the results of the research study?

We aim to publish the results in a scientific journal and present them at scientific meetings. Your details will remain strictly confidential, with no personal information being included in any publications.

Who has reviewed the study?

This type of research is looked at by an independent group of people called a Research Ethics Committee, who protect your safety, rights, wellbeing and dignity. This study has been reviewed by the Royal College of Surgeons in Ireland (RCSI) Research Ethics Committee.

What if there is a problem?

If you have any queries about this study or if you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should firstly contact the study co-ordinator or study supervisor on the details below:

Dr. Muireann de Paor (study co-ordinator),

HRB Centre for Primary Care Research,

Beaux Lane House,

Mercer St. Lower,

Dublin 2.

01 4028604

Prof. Tom Fahey (study supervisor),

HRB Centre for Primary Care Research,

Beaux Lane House,

Mercer St. Lower,

Dublin 2.

01 4022305

RCSI has insurance arrangements in place to provide for harm arising from participation in the study.

Thank you for taking the time to read this information sheet.

Appendix 7: Participant Information Sheet, short version

HRB CENTRE FOR PRIMARY CARE RESEARCH DEPARTMENT OF GENERAL PRACTICE

Division of Population Health Sciences Royal College of Surgeons in Ireland

Beaux Lane House, Mercer Street Lower, Dublin 2, Ireland

Tel +353 1 4022473 **Fax** +353 1 4022764

Email enquiries.cpcr@rcsi.ie **www**.hrbcentreprimarycare.ie

Research Study on Infectious Mononucleosis (Glandular Fever)

Summary Information Leaflet

We want to find out more about infectious mononucleosis (glandular fever) which is a viral infection that causes sore throat, swollen lymph glands and sometimes other symptoms.

We will be examining which symptoms best predict a diagnosis of actual glandular fever. We will be developing a prediction rule in which researchers try to identify the best combination of medical signs and symptoms in predicting the probability of glandular fever. In addition, we want to find out how many people who test positive for glandular fever will end up missing time from college or usual activities or will end up having symptoms over a longer period. The final aim of our study is to test an established prediction rule for a bacterial cause of sore throat and see how that performs in an Irish setting.

This is not a clinical trial involving medication. Your GP will continue to provide care for you during your illness in the usual way.

You have been invited to take part because you or they have come to see your GP or nurse with glandular fever type symptoms. Your doctor will assess whether you are eligible for the study. If you **want to** take part your consent will be taken for this.

This is what will happen if you decide to take part:

The trial will be explained to you

Your eligibility will be checked

You will give your consent to take part

You will fill out a questionnaire and a form with your contact details (so that the researchers can contact you)

Your GP or a GP from the study team will examine you and complete the baseline form

Two blood samples and a throat swab will be taken for testing

The research team will contact you after three months and again at six months to ask you a few questions about your illness

You will be entered into a prize draw for a 250 euro One4all gift voucher

---


RCSI
NUI Galway
OÉ Gaillimh

Queen's University
Belfast

Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

Appendix 8: Participant Consent Form

HRB CENTRE FOR PRIMARY CARE RESEARC

DEPARTMENT OF GENERAL PRACTICE

Division of Population Health Sciences Royal College of Surgeons in Ireland

Beaux Lane House, Mercer Street Lower, Dublin 2, Ireland

Tel +353 1 4022473 **Fax** +353 1 4022764

Email enquiries.cpcr@rcsi.ie **www**.hrbcentreprimarycare.ie

		PLEASE INITIAL
1.	I confirm I have read and understood the information sheet for the above study and have had the opportunity to ask questions.	
2.	I understand my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected.	
3.	I understand that a blood sample and a throat swab will be taken to be used by the research team for analysis. I consider this sample a gift to the Royal College of Surgeons in Ireland (RCSI) and I understand I will not gain any direct personal benefit from this.	
4.	I understand that I will be required to provide information to the research team through online / paper and telephone questionnaires.	
5.	I consent to being contacted by the research team for the purposes of study follow up and I understand that this will require me to provide the research team with my contact details	
6.	I understand that my medical notes, and my laboratory results, including information about any visits to hospital (in relation to this illness only) may be reviewed and data collected by the research team. I permit these individuals access to my clinical records.	
7.	I understand that data collected during the study may be looked at by authorised individuals from RCSI, regulatory authorities and authorised people for research purposes. I permit these individuals access to my research records.	
8.	I agree to take part in the above study.	

SORE THROAT STUDY CONSENT FORM

Name of Person Taking Consent (Print) Date Signature

Name of Participant (Print) Date Signature

What if there is a problem?

If you have any queries about this study or if you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should firstly contact the study co-ordinator or study supervisor on the details below:

Dr. Muireann de Paor (study co-ordinator),

HRB Centre for Primary Care Research,

Beaux Lane House,

Mercer St. Lower,

Dublin 2.

01 4028604

Prof. Tom Fahey (study supervisor),

HRB Centre for Primary Care Research,

Beaux Lane House,

Mercer St. Lower,

Dublin 2.

01 4022305

Participating institutions in the HRB Centre for Primary Care Research are:

RCSI NUI Galway OÉ Gaillimh Queen's University Belfast Trinity College Dublin Coláiste na Tríonóide, Baile Átha Cliath The University of Dublin

Appendix 9: Participant Questionnaire

HRB CENTRE FOR PRIMARY CARE RESEARCH DEPARTMENT OF GENERAL PRACTICE

Division of Population Health Sciences Royal College of Surgeons in Ireland

Beaux Lane House, Mercer Street Lower, Dublin 2, Ireland

Tel +353 1 4022473 **Fax** +353 1 4022764

Email enquiries.cpcr@rcsi.ie **www**.hrbcentreprimarycare.ie

Patient questionnaire: Infectious Mononucleosis Study

Participant ID:	__ __ / __ __ / __ __ __ __
(from practice software database; e.g., Socrates, Helix)	
Date of Recruitment:	__ __ / __ __ / __ __ __ __

1 BASIC DEMOGRAPHICS

Age:

Gender

Male	
Female	

Past medical history: please write in any medical history of note for example asthma, diabetes, any surgery that you have had etc.

Number of consultations with your college doctor or own GP in past six month

2 SYMPTOMS

Please tick yes or no for presence or absence of symptoms

	Yes	**No**
Cough		
Nasal congestion, runny nose		

Sore throat		
Shortness of breath		
Fever		
Headache		
Muscle aches and / or pains		
Sweats / chills		
Fatigue		
Not sleeping well		
Dizziness		
Feeling generally unwell		
Poor appetite		

3 FATIGUE SCORE

FATIGUE SEVERITY SCALE (FSS). Please circle the number between 1 and 7 which you feel best fits the following statements. This refers to your usual way of life **within the last week**. 1 indicates "strongly disagree" and 7 indicates "strongly agree."

Read and circle a number.	**Strongly Disagree →Strongly Agree**
1. My motivation is lower when I am fatigued.	1 2 3 4 5 6 7
2. Exercise brings on my fatigue.	1 2 3 4 5 6 7
3. I am easily fatigued.	1 2 3 4 5 6 7
4. Fatigue interferes with my physical functioning.	1 2 3 4 5 6 7
5. Fatigue causes frequent problems for me.	1 2 3 4 5 6 7
6. My fatigue prevents sustained physical functioning.	1 2 3 4 5 6 7
7. Fatigue interferes with carrying out certain duties and responsibilities.	1 2 3 4 5 6 7
8. Fatigue is among my most disabling symptoms.	1 2 3 4 5 6 7
9. Fatigue interferes with my work, family, or social life.	1 2 3 4 5 6 7

VISUAL ANALOGUE SCALE FOR GLOBAL FATIGUE: Please mark an "X" on the number line which describes your global fatigue at the moment with 0 being normal and 10 being the most fatigued.

0 1 2 3 4 5 6 7 8 9 10

What if there is a problem?

If you have any queries about this study or if you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should firstly contact the study co-ordinator or study supervisor on the details below:

Dr. Muireann de Paor (study co-ordinator),

HRB Centre for Primary Care Research,

Beaux Lane House,

Mercer St. Lower,

Dublin 2.

01 4028604

Prof. Tom Fahey (study supervisor),

HRB Centre for Primary Care Research,

Beaux Lane House,

Mercer St. Lower,

Dublin 2.

01 4022305

Participating institutions in the HRB Centre for Primary Care Research are:

Appendix 10: Case Report Form (CRF)

1 **ELIGIBILITY**

Inclusion

Yes	No	
		Participant aged over 18
		Presenting with sore throat and at least one other of the following symptoms: malaise, fatigue, lymphadenopathy, fever, headache
		Symptom duration 7 days or less
		Is willing and able to give informed consent and comply with all trial requirements

Exclusion

Yes	No	
		Condition or treatment associated with significant impaired immunity
		Health literacy or language difficulties sufficient to cause difficulty understanding the study information

If all answers to **A** are **YES** and all answers to **B** are **NO** then the participant is **Eligible** to be recruited

If so please continue on with recruitment

If patient not willing to participate please state why (e.g. time pressure, not willing to have bloods taken etc.) and just return this page to Muireann

CONSENT MUST BE TAKEN BEFORE ANY FURTHER TRIAL PROCEDURES CAN TAKE PLACE

Participant ID (from practice software database; e.g., __ __ __ __ __

Socrates, Helix):

Date of Recruitment: __ __ / __ __ / __ __ __ __

2 **CLINICAL EXAMINATION**

Temperature

Pulse

Respiratory rate

Throat examination

Presence of exudate on pharynx

Yes	
No	

Presence of exudate on tonsils

Yes	
No	

Presence of palatal petechiae

Yes	
No	

Lymph node examination

Presence of enlarged (>1cm approx.) anterior cervical lymph nodes

Yes	
No	

Presence of tender anterior cervical lymph nodes

Yes	
No	

Presence of enlarged (>1cm approx.) or tender posterior cervical lymph nodes

Yes	
No	

Presence of enlarged (>1cm approx.) or tender inguinal lymph nodes. ONLY PERFORM THIS EXAMINATION IF FEASIBLE

Yes	
No	

Presence of enlarged (>1cm approx.) or tender axillary lymph nodes. ONLY PERFORM THIS EXAMINATION IF FEASIBLE

Yes	
No	

Abdominal examination

Presence of splenomegaly

Yes	
No	

Presence of hepatomegaly

Yes	
No	

3 **CARE ADVICE**

What advice did you give to the participant about how to care for themselves?

Pharmacological management:

A. ☐ Take paracetamol at regular intervals

B. ☐ Take paracetamol when required

C. ☐ Take ibuprofen at regular intervals

D. ☐ Take ibuprofen when required

E. ☐ Take other pain medication when required

F. ☐ Antibiotic prescription given

G. ☐ Delayed script given for antibiotic

Other advice:

H. ☐ Rest in bed

I. ☐ Take time off school or work

J. ☐ Take time of school or work for a set number of days

Number of days stated:_____

K. ☐ Other:

_____

4

TENTATIVE DIAGNOSIS

Please write in what you think the differential diagnosis might be

Appendix 11: Fatigue Severity Scale (FSS)

Choose a number from 1 to 7 that indicates your degree of agreement with the following statements where **1 indicates strongly disagree** and **7 indicates strongly agree**. Please answer the questions with reference to how you have been feeling **on average over the last week**.

	Strongly disagree						**Strongly agree**
1. My motivation is lower when I am fatigued	1	2	3	4	5	6	7
2. Exercise brings on my fatigue	1	2	3	4	5	6	7
3. I am easily fatigued	1	2	3	4	5	6	7
4. Fatigue interferes with my physical functioning	1	2	3	4	5	6	7
5. Fatigue causes frequent problems for me	1	2	3	4	5	6	7
6. My fatigue prevents sustained physical functioning	1	2	3	4	5	6	7
7. Fatigue interferes with carrying out certain duties and responsibilities	1	2	3	4	5	6	7
8. Fatigue is among my three most disabling symptoms	1	2	3	4	5	6	7
9. Fatigue interferes with my work, family or social life	1	2	3	4	5	6	7

Appendix 12: Visual Analogue Scale for Global Fatigue

VISUAL ANALOGUE SCALE FOR GLOBAL FATIGUE : Please mark an "X" on the number line which describes your global fatigue at the moment with 0 being normal and 10 being the most fatigued.

0 1 2 3 4 5 6 7 8 9 10