Management of Infectious Mononucleosis

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Infectious mononucleosis (IM) is a constellation of clinical features largely seen in adolescence and young adults. It is typified by pharyngitis, prominent cervical lymphadenopathy and high fevers. It therefore can be difficult to distinguish the disease from the far more common presentation of bacterial tonsillitis (BT). Epstein Barr Virus (EBV) causes IM in the majority of cases, and thus a distinguishing feature is an associated lymphocytosis, rather than the neutrophilia seen in BT. Along with the different aetiology and diagnostic pathways, there are a number of aspects of the disease that warrant further research. This thesis aims to investigate the epidemiology, diagnosis and management of the disease, as well as providing a review of a number of areas of controversy.

IM is associated with a high lymphocyte count and recent publications have sought to exploit this as a screening tool. I examined 1000 laboratory results but found that the lymphocyte to white cell count alone was not sufficient to act as a screening tool in IM.

IM tends to present as a more severe infection than BT, but there was no literature to collaborate what many physicians had noted. The admission rates to a tertiary hospital of both diseases were examined over a 20-year period. This allowed us to show that IM had a significantly longer hospital stay, providing evidence for the first time of the severity of the disease.

As 90% of cases are viral in origin, antibiotics would unsurprisingly not usually have a role in the treatment of IM. However, the virally driven pharyngitis can lead to
anaerobic overgrowth in the oral cavity contributing to the severity of the symptoms. I carried out a randomised controlled trial to demonstrate a significant length of stay decrease in those treated with anaerobic antibiotics.

Finally a comprehensive review of multiple controversial topics associated with IM was undertaken. I discussed subjects such as the association of IM with Multiple Sclerosis (MS), chronic fatigue, splenic rupture, and relationship with malignancies and treatment with steroids.

Findings from this thesis may have important clinical implications in the epidemiology, diagnosis and management of infectious mononucleosis.
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PRESENTATION OF THESIS

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Table 1: Journal publication, target audience and ISI impact factor for each chapter presented in this thesis.

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GLOSSARY

Aetiology - the cause or set of causes, or manner of causation of a disease or condition.

Anaerobic bacteria - are bacteria that do not live or grow when oxygen is present. In humans, these bacteria are most commonly found in the gastrointestinal tract.

Blinded study - A study done in such a way that the patients or subjects do not know (is blinded as to) what treatment they are receiving to ensure that the results are not affected by a placebo effect (the power of suggestion).

Cervical - of the neck

Chronic fatigue syndrome - is a debilitating and complex disorder characterized by profound fatigue that is not improved by bed rest and that may be worsened by physical or mental activity.

Control group - is a baseline group that receives no treatment or a neutral treatment. To assess treatment effects, the experimenter compares results in the treatment group to results in the control group.

Cytomegalovirus - a kind of herpesvirus that usually produces very mild symptoms in an infected person but may cause severe neurological damage in people with weakened immune systems and in the newborn.
Epidemiology - is the study and analysis of the patterns, causes, and effects of health and disease conditions in defined populations.

Eponymous - relating to, or being the person or thing for whom or which something is named.

Haematology - is the study of the morphology and physiology of blood.

Heterophile antibodies - are antibodies produced against poorly defined antigens. These are generally weak antibodies with multispecific activities.

Hypertrophy - Hypertrophy is the increase in the volume of an organ or tissue due to the enlargement of its component cell.

Hypothesis - a supposition or proposed explanation made on the basis of limited evidence as a starting point for further investigation.

Incidence - is a measure of the probability of occurrence of a given medical condition in a population within a specified period of time.

Incubation period - the period between exposure to an infection and the appearance of the first symptoms.

Lymphadenopathy - Enlargement/ disease of the lymph glands.

Lymphocytosis - an increase in the number of lymphocytes (type of white blood cell).
Mean- average of a set of values

**Median**- denoting or relating to a value or quantity lying at the midpoint of a frequency distribution of observed values or quantities, such that there is an equal probability of falling above or below it.

**Monospot** – (mononuclear spot test)a form of the heterophile antibody test, is a rapid test for infectious mononucleosis due to Epstein–Barr virus (EBV).

**Multiple Sclerosis**- A chronic autoimmune disorder affecting movement, sensation, and bodily functions. It is caused by destruction of the myelin insulation covering nerve fibers (neurons) in the central nervous system (brain and spinal cord).

**Neutrophilia**- an elevated level of neutrophils (type of white blood cell)

**Nonparametric**- statistics not based on parameterized families of probability distributions. They include both descriptive and inferential statistics

**Peritonsillar abscess (Quinsy):** A collection of pus behind the tonsils that pushes one of the tonsils toward the uvula

**Petechiae**- a small red or purple spot caused by bleeding into the skin.

**Pharyngitis**- Inflammation of the pharynx/sore throat
Polymerase chain reaction- is a process used in molecular biology to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Positively skewed- is a measure of the asymmetry of the probability distribution of a real-valued random variable about its mean. The skewness value can be positive or negative, or even undefined.

Primary endpoints- measure outcomes that will answer the primary (or most important) question being asked by a trial, such as whether a new treatment is better at preventing disease-related death than the standard therapy.

Randomization- A method based on chance alone by which study participants are assigned to a treatment group. Randomization minimizes the differences among groups by equally distributing people with particular characteristics among all the trial arms.

Sensitivity is the ability of a test to correctly identify those with the disease (true positive rate)

Specificity- is the ability of the test to correctly identify those without the disease (true negative rate).

Sequelae- a condition that is the consequence of a previous disease or injury
Standard deviation- is a measure that is used to quantify the amount of variation or dispersion of a set of data values.

Subclinical- relating to or denoting a disease that is not severe enough to present definite or readily observable symptoms.

Ubiquitous- Present everywhere.
ABBREVIATIONS

- **BMJ**- British Medical Journal
- **BT**- Bacterial tonsillitis
- **CDC**- Centre for disease control
- **CONSORT**- (CONsolidated Standards of Reporting Trials)
- **CMV**- Cytomegalovirus
- **EBV**- Epstein Barr Virus
- **HIPE**- Hospital In-Patient Enquiry
- **HIV**- Human Immunodeficiency Virus
- **IgG**- Immunoglobulin G
- **IgM**- Immunoglobulin M
- **IM**- Infectious Mononucleosis
- **L/WCC**- Lymphocyte to White Cell Count ratio
- **NHS**- National Health Service
- **SD**- Standard deviation
- **WCC**- White Cell Count
INTRODUCTION

Infectious Mononucleosis (IM) is a well-recognized disease process, which mainly affects teenagers and young adults. A number of controversies remain in the diagnosis, treatment and management of potential sequelae of IM. Filatov's disease, Pfeiffer’s disease, glandular fever, Turk’s lymphomatosis, Sprunt’s disease, and the kissing disease are all synonyms of IM. Each designation gives us an insight into the evolution of our understanding of the disease. Nil Filatov, a prominent 19th century physician, considered to be the father of Russian paediatrics, is credited with the first description on IM in 1887. He used the name “idiopathic denitis” (Filatov 1887). Just two years later a German physician, Emil Pfeiffer, described “Drusenfieber”, or glandular fever, independently from Filatov (Pfeiffer 1889). His description has been portrayed as a more comprehensive account of the clinical symptoms and signs that we now recognize in those with IM (Evans 1974). Wilhelm Türk was born in 1871 in Silesia in modern day Poland, then part of the Austrian empire. He was a renowned haematologist credited with many innovations in what was then an emerging field. He gave an excellent description of the morphology of the blood and course of disease, which would only later would be recognised as IM (Lehndorff 1954). The last of the eponymous titles fittingly also gave the disease the name by which we know it today. Thomas P. Sprunt and Frank A. Evans published a paper in 1920 clearly describing the clinical and haematological picture in a group of students attending Johns Hopkins University and for the first time used the term Infectious Mononucleosis (Sprunt and Evans 1920). The Paul-Bunnell test or cold agglutination test was discovered serendipitously when Dr Bunnell, working as a research assistant to Dr John Paul, left
Introduction

an incubation tray (investigating a link between serum sickness and rheumatic fever) in a fridge overnight. The heterophile antibodies were greatly increased by the cold and the serum sample was traced back to a patient with IM (Evans 1974, Paul and Bunnell 1982). This was further improved upon by using horse red cell (instead of sheep red cells), for increased accuracy and speed and became known as the monospot test (Lee et al. 1968). The aetiology of the disease remained unclear, even after many young medical students were inoculated with the disease(Evans 1950, Niederman and Scott 1965). The eponymous Epstein Barr virus (EBV) was discovered in 1964 by doctors working on the newly diagnosed Burkitt’s disease(Epstein et al. 1964). However it took another act of fortune to link the two. A laboratory technician working with Werner and Gertrude Henle in 1968 on the EBV virus in Philadelphia became unwell and was diagnosed with IM. A comparison of serum samples collected from her before and after the onset revealed development of antibodies to EBV (Henle et al. 1968). The association was confirmed with a number of prospective studies in the subsequent years (Niederman et al. 1970, Sawyer et al. 1971). And the final synonym- Humans are the only source of the infection, and transmission occurs primarily through saliva, hence its popular label as “the kissing disease” (Guo et al. 2010).

There is still no evidence based or consensus guidelines for the diagnosis of the disease(Ebell 2004). The Hoagland criteria, from a study of 500 patients with IM, are the most widely quoted guidelines in the diagnosis of IM. This report states that a patient presenting with fever, pharyngitis, and lymphadenopathy, with at least a 50% lymphocytosis, a 10% minimum of which are atypical, and confirmed by positive serological test can be diagnosed with IM (Hoagland 1975). The heterophile test may be falsely negative in up to 25% of adults, or up to 50% of young children(Linderholm
et al. 1994), in the first week of symptoms, when antibody levels are below the limit of detection of the assay (Hoagland 1975). If strongly suspected, the monospot test may be repeated after a week (Pagana et al. 2010). Some recent papers have suggested that the lymphocyte to white cell count (L/WCC) ratio of 0.35 (Wolf et al. 2007) or an elevated absolute lymphocyte count (>4x10⁹/L) (Biggs 2011, Biggs et al. 2013) could be used as a ‘quick reference tool’ or ‘quickly available alternative test for the detection of glandular fever’. The aim of the first chapter of this thesis was to assess the validity of these results in a larger matched cohort of patients. The diagnosis of IM was investigated using a retrospective analysis of the lymphocyte count of patients who have been diagnosed with IM with a positive monospot test. We compared the lymphocyte to white cell count ratio of 500 patients with a positive result to 500 patients with negative monospot results. All patients were adults, with paediatric patients excluded. The patients were selected from a database from within the department of haematology.

EBV is one of the most common viral infections in human beings (Masucci and Ernberg 1994). In childhood, the disease is usually subclinical, and most of the population will have acquired immunity by adolescence (Schuster and Kreth 1992). However infection of adolescents or adults results in IM in 30 to 70% of cases and can prove severe (Tattevin et al. 2006). EBV infection in childhood is associated with low socioeconomic status, poor hygiene and crowding so that improved housing and decreasing family sizes may have resulted in a change in the epidemiology of EBV and therefore infectious mononucleosis (Crowcroft et al. 1998). Studies have shown that its acquisition is delayed in more affluent social classes resulting in increased hospital admissions (Morris and Edmunds 2002). Anecdotally IM is a more severe infection
than bacterial tonsillitis (BT) requiring a longer stay in hospital, however there is no evidence in the literature to support this. The aim of the second part of this thesis was to perform an epidemiological review of IM over a 20 years period, in order to compare the length of stay in hospital of IM against a cohort of patients diagnosed with BT. The Hospital In-Patient Enquiry (HIPE) system was utilised to identify patients. All patients that had been coded for emergency admissions for IM were included. Those that have been coded for Cytomegalovirus (CMV) or Mononucleosis syndrome were excluded. Only patients over 15 were included. We compared this data to patients admitted with acute tonsillitis. Those with peritonsillar abscess (quinsy) and post tonsillar bleeds were excluded. Incidence and length of were evaluated. Data was analysed by Dr Saunders at the Statistical Consulting unit in the University of Limerick.

As predominantly a viral illness, treatment of IM is generally supportive, but a number of different treatment modalities have been examined over the years. Antiviral treatment with acyclovir has been found to have a positive effect (Andersson et al. 1986), and to be useful in severe cases of airway compromise (Andersson et al. 1987). However their routine use is not currently recommended. A less obvious treatment option is that of metronidazole and tinidazole, anaerobic antibacterial agents. EBV exerts a transient suppression of immunoglobulin-coating of bacteria harboured on the tonsillar surfaces, with consequent abundant bacterial attachment to the epithelial cells and massive bacterial colonization on the palatine tonsils and penetration into the epithelial cells (Brook and de Leyva 1994, Stenfors et al. 2001). It has been suggested that metronidazole may help to hasten recovery in IM by suppression of the oral anaerobic flora that might otherwise contribute to the inflammatory process (Brook and Deleyva 1996, Brook 2005). A number of small studies were conducted in both
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metronidazole (Hedstrom et al. 1978, Davidson et al. 1979, Hedstrom 1980, Davidson et al. 1982, Dalmau et al. 1990) and tinidazole (Marklund et al. 1984, Marklund et al. 1986), which demonstrated a positive therapeutic effect of the antibiotics. However these studies did not have sufficient numbers to give statistical weight to a recommendation of routine metronidazole use, and therefore there is ongoing controversy. The aim of the third part of the study is to confirm or reject the hypothesis that Metronidazole is of use in the treatment of IM. A randomised controlled trial was therefore undertaken in University Hospital Limerick to evaluate the efficacy of the antibiotic metronidazole in the treatment of IM. In one arm of the study, patients were treated with Benzylpenicillin and Metronidazole and the control arm with Benzylpenicillin only, which is the standard of care. Patients given steroids or other antibiotics, due to penicillin allergy, were excluded. Daily markers of infection and inflammation were observed. Adult (Over 15 years of age) patients were randomised on a one is to one basis from throughout 2010-2011. These patients were given information on the study and consented to take part in the study. The patients were blinded to the medication. We assessed the patients daily with a symptom questionnaire and also with daily examinations. The primary outcome was length of stay in the hospital.

The fourth and final part of the study was a review of controversial topics in IM. The review aims to highlight these areas where there has been recent developments, including diagnosis, the association with multiple sclerosis and chronic fatigue, treatment of the disease including steroid use, associations with malignancies and at what time to return to sport. The review was aimed primarily at General Practitioners to assist them in some of the more difficult of contentious topics that could arise when
treating patients with IM, particularly in the era of easy access to medical literature via the Internet. This review also acts as a summary of the research that was undertaken for this thesis it was published after the first three papers, which are all cited in the paper. The review also examines possible future areas of research. In a supplementary section, four further publications were added that arose as a direct result of the principal papers in this Thesis. These included three letters to the Editor and an invited commentary of a systematic review and meta-analysis. I felt that these should be included not only to further the work that has already been published but also to demonstrate the impact of this work.

The overall aim of the project is to further our knowledge of the management of infectious mononucleosis.
CHAPTER 1

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

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Clinical Otolaryngology: PMID 21108750

Conception and Design: PL, JPON, JEF

Analysis and Interpretation: PL, JPON, JEF, TOD

Data Collection: PL

Writing the article: PL, JPON,

Critical Revision of article: JEF, TOD
ABSTRACT

Objective: We investigated the hypothesis that a Lymphocyte/White Cell Count ratio should be used as a diagnostic indicator of Infectious Mononucleosis

Design: Retrospective study to compare lymphocyte counts and white blood cell counts, against the criterion standard, the mononucleosis spot test.

Setting: Department of Otolaryngology, Mater Misericordiae University Hospital, Dublin, Ireland.

Participants: We reviewed 1000 patients who had Monospot assays, 500 positive and 500 negative.

Main Outcome Measures: The lymphocyte counts and white blood cell ratio was calculated and compared with the monospot result to calculate the sensitivity and specificity at various ratios.

Results: The lymphocyte counts and white blood cell ratio was significantly different in the positive and negative monospot groups ($P<0.05$). The mean lymphocyte counts and white blood cell ratio in the positive group was 0.49 and the mean L/WCC ratio in the monospot negative group was 0.29. A ratio of 0.35 had a specificity of 72.2% and a sensitivity of 84% for the detection of glandular fever. A higher ratio will give a greater specificity, but a lower sensitivity, and visa versa.
Conclusions: The lymphocyte to white cell ratio is not sufficient to diagnose or exclude Infectious Mononucleosis. Thus this study does not confirm the conclusions of earlier studies.
Chapter 1  Lymphocyte to WCC ratio

1.1 Introduction

Infectious mononucleosis was first described as a clinic entity in 1889 when the term glandular fever was applied to the triad of pharyngitis, fever, and lymphadenopathy (Pfeiffer 1889). The first definition of infectious mononucleosis was made in 1920 when it was discovered that a number of patients with glandular fever had similar blood films. These demonstrated an absolute lymphocytosis, with abnormally abundant cytoplasm in mononuclear cells (Sprunt and Evans 1920). The heterophile antibody test became the basis for serologic diagnosis of infectious mononucleosis, when in 1932 Paul and Bunnell discovered that serum from patients with infectious mononucleosis caused sheep erythrocytes to agglutinate (Davidsohn 1937). It was not until 1968 that the then newly discovered Epstein-Barr virus was identified as the cause of infectious mononucleosis (Henle et al. 1968). However today, there is still no evidence based or consensus guidelines for the diagnosis of the disease (Ebell 2004).

Infectious mononucleosis is associated with a high white cell count with a relative lymphocytosis and the diagnosis is usually confirmed by a positive monospot test (Hoagland 1975). This is the basis for diagnosis in the National Health Service (NHS) clinical knowledge summary, a resource for primary care physicians in the UK, on infectious mononucleosis (Clinical Knowledge Summaries 2011). It has been suggested recently that the lymphocyte to white cell count (L/WCC) ratio could be a quickly available alternative test for the detection of glandular fever (Wolf et al. 2007). The L/WCC of a series of patients with glandular fever was compared with that of a relatively similar number of patients with bacterial tonsillitis and it was concluded that a L/WCC ratio higher than 0.35 had a sensitivity of 90% and a specificity of 100% for the detection of glandular fever. This indicated a better specificity and sensitivity than
the mononucleosis spot test. The aim of our study was to assess the validity of these results in a larger matched cohort of patients.
1.2 Methods

1.2.1 Ethical considerations

As these data are collected routinely as part of the clinical care of these patients and analyzed retrospectively, no patient records or extra patient data were accessed for this study. Spreadsheets of data of patients that had had monospot tests were acquired from the haematology department and the L/WCC ratio was compared for patients with positive and negative results. All patient records were anonymised prior to analysis. Therefore no ethical approval was deemed to be required.

1.2.2 Data Gathering and analysis

1000 monospot tests in patients with tonsillitis both in an outpatient and inpatient setting were analysed to compare the L/WCC ratio in 500 positive and 500 negative results. All monospot and white cell count differentials were carried out within the department of haematology, the Mater Misericordiae University Hospital, Dublin. Data was collated using Excel software, and analysed using cross tabulation, independent sample t-test and ANOVA tests.

1.2.3 The Monospot test

The monospot tests were performed with “Clearview IM” detection kits. The Clearview Mono test is a qualitative membrane strip based immunoassay for the detection of infectious mononucleosis heterophile antibodies in whole blood. In this test procedure, bovine erythrocyte extracted antigen is coated on the test line region of the strip. The sample reacts with bovine erythrocyte extracted antigen coated particles that have been applied to the label pad. This mixture migrates chromatographically along the length of the test strip and interacts with the coated bovine erythrocyte extracted antigen. If the
sample contains infectious mononucleosis antibodies, a coloured line will appear in the test line region indicating a positive result. If the sample does not contain IM heterophile antibodies, a coloured line will not appear in this region indicating a negative result. To serve as a procedural control, a coloured line will always appear at the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred (Clearview IM product information 2010).
1.3 Results

The distribution of the participants by gender and test results is presented in Table 3. Overall, there were 42.4% males and 57.6% females in the survey.

**Table 3: Patient Demographics**

<table>
<thead>
<tr>
<th></th>
<th>Positive test</th>
<th>Negative test</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>255</td>
<td>169</td>
<td>424 (42.4%)</td>
</tr>
<tr>
<td>Female</td>
<td>245</td>
<td>331</td>
<td>576 (57.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>500</td>
<td>1000</td>
</tr>
</tbody>
</table>

We can see from Table 4 that the majority of the patients (more than 70% from each group) are outside this range. We see that most of the positive cases are above the 40% range and most of the negative cases are below 35% range. So it can be initially inferred that the critical L/WCC ratio will lie somewhere in the range 35% to 40%.

**Table 4: Patient demographics and L/WCC ratio categories**

<table>
<thead>
<tr>
<th></th>
<th>&lt;25%</th>
<th>25%&lt; and &lt;35%</th>
<th>35%&lt; and &lt;40%</th>
<th>40%&lt; and &lt;50%</th>
<th>50%&lt;</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive female</td>
<td>22</td>
<td>27</td>
<td>24</td>
<td>51</td>
<td>121</td>
<td>245</td>
</tr>
<tr>
<td>Positive male</td>
<td>16</td>
<td>15</td>
<td>26</td>
<td>59</td>
<td>139</td>
<td>255</td>
</tr>
<tr>
<td>Negative female</td>
<td>102</td>
<td>133</td>
<td>48</td>
<td>38</td>
<td>10</td>
<td>331</td>
</tr>
<tr>
<td>Negative male</td>
<td>54</td>
<td>75</td>
<td>20</td>
<td>17</td>
<td>3</td>
<td>169</td>
</tr>
<tr>
<td>Total</td>
<td>194</td>
<td>250</td>
<td>118</td>
<td>165</td>
<td>273</td>
<td>1000</td>
</tr>
</tbody>
</table>
There are two independent groups, one with positive monospot test and the other with negative monospot test. In order to compare the mean L/WCC ratio for these two groups we will use t-test. The P-P plot can be used to test the normality of the dependent variable (if the observed values are close to the expected values, close to linearity, the data will be considered normally distributed). The P-P plot of L/WCC ratio for the two groups is shown in Figure 1. It is clear from Figure 1 that the data is normally distributed for the two groups and we can safely say that the assumptions for using t-test are all satisfied.

![Normal P-P plot for L/WCC ratio](image)

**Figure 1: Normal P-P plot for the L/WCC ratio**

Table 5 shows descriptive statistical results for L/WCC ratio for the two independent groups. We can clearly see the difference in the mean values of the two groups. The t-test was significant ($t=24.797$, $P=0.00 < 0.05$) which shows that there is significant difference in L/WCC ratio between the two groups.
Table 5: Descriptive statistics for L/WCC ratio

<table>
<thead>
<tr>
<th>Test result</th>
<th>Number</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>L/WCC ratio</td>
<td>Positive</td>
<td>500</td>
<td>0.4927</td>
<td>0.148088</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>500</td>
<td>0.2934</td>
<td>0.102499</td>
</tr>
</tbody>
</table>

For different values of L/WCC ratio we get different values of sensitivity and specificity. We see that as the sensitivity increases the specificity decreases and for an L/WCC ratio of 0.376 both the sensitivity and specificity are equal (approx. 80%). Table 6 gives a detailed analysis of sensitivity and specificity at different L/WCC ratio.

Table 6: Sensitivity and Specificity for different L/WCC ratios

<table>
<thead>
<tr>
<th>L/WCC ratio</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive percentage</th>
<th>Negative predictive percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>100.00%</td>
<td>00.80%</td>
<td>50.20%</td>
<td>100.00%</td>
</tr>
<tr>
<td>0.1</td>
<td>99.00%</td>
<td>03.80%</td>
<td>50.71%</td>
<td>79.16%</td>
</tr>
<tr>
<td>0.2</td>
<td>95.20%</td>
<td>18.60%</td>
<td>53.90%</td>
<td>79.48%</td>
</tr>
<tr>
<td>0.30</td>
<td>88.60%</td>
<td>50.20%</td>
<td>64.01%</td>
<td>81.49%</td>
</tr>
<tr>
<td>0.35</td>
<td>84.00%</td>
<td>72.20%</td>
<td>75.13%</td>
<td>81.85%</td>
</tr>
<tr>
<td>0.376</td>
<td>80.40%</td>
<td>80.20%</td>
<td>80.23%</td>
<td>80.36%</td>
</tr>
<tr>
<td>0.40</td>
<td>74.00%</td>
<td>86.00%</td>
<td>84.09%</td>
<td>76.78%</td>
</tr>
<tr>
<td>0.45</td>
<td>65.00%</td>
<td>93.00%</td>
<td>90.27%</td>
<td>72.65%</td>
</tr>
<tr>
<td>0.50</td>
<td>52.00%</td>
<td>97.40%</td>
<td>95.23%</td>
<td>66.98%</td>
</tr>
</tbody>
</table>
The distribution of L/WCC ratio for the two independent groups is shown in Figure 2. As mentioned in Table 2b, for a L/WCC ratio of 0.376 the sensitivity and specificity equal and approximate to 80%. If we increase or decrease the ratio one of these parameters will increase and the other will decrease. If we go for a higher sensitivity we can see from the table that we have a lower specificity and vice-versa. Thus we have optimized the sensitivity and specificity to calculate the present.

**Figure 2: Graphical representation of L/WCC ratio in all patients**
1.4 Discussion

1.4.1 Synopsis of key findings

Our findings demonstrate there is a significant difference between the lymphocyte count in patients with infectious mononucleosis and those with negative monospot tests. An increased lymphocyte count is associated with a positive monospot results and using a L/WCC ratio of 0.35 or greater had a specificity of 72% a sensitivity of 84% for the detection of glandular fever. A higher ratio will give a greater specificity, but a lower sensitivity, and visa versa.

1.4.2 Strengths and weaknesses of the study

The foremost strength of this study is the number of monospot results assessed. It is comparable to Hoagland’s research from 1975 and has more than 4 times the patients in the positive cohort and 5 times the patients in the negative cohort of the more recent Wolf study. The Clearview monospot test was used in all cases, as an appropriate reference standard, which has been described in the methods section. Verification bias is avoided, as all tests were carried out in the same laboratory with the same detection kits. The laboratory technicians were essentially blinded, as this was a retrospective study. The weaknesses of paper include the fact that the study was carried out with only laboratory data without any clinical correlation and included both inpatient and outpatient results. There is a possibility therefore that some patients’ results were analysed that should have been excluded.
1.4.3 Comparisons with other studies

The objective of this study was to compare our results to a recent paper by Wolf et al, however our findings are more in line with an earlier paper by Hoagland (Hoagland 1975). The Hoagland criteria, from a study of 500 patients with infectious mononucleosis, are the most widely quoted guidelines in the diagnosis of infectious mononucleosis (Hoagland 1975). This report states that a patient presenting with fever, pharyngitis, and lymphadenopathy, with at least a 50% lymphocytosis, a 10% minimum of which are atypical and confirmed by positive serological test can be diagnosed with infectious mononucleosis (Hoagland 1975, Ebell 2004). Wolf et al recommended that the L/WCC alone could be used to act as a quickly available alternative test for the detection of glandular fever (Wolf et al. 2007). They analysed 120 patients with glandular fever and 100 with bacterial tonsillitis and reported that a L/WCC ratio higher than 0.35 had a sensitivity of 90% and a specificity of 100% for the detection of glandular fever. However, 0.35 is marginally above the normal range of Lymphocyte to white cell count ratio, which is 0.25-0.33 (Aghenta et al. 2008). Our results with much larger numbers showed a sensitivity of 84% and specificity of 72.2% for a ratio of 0.35. A higher ratio will give a greater specificity, but a lower sensitivity, and visa versa. They also added that the specificity and sensitivity of this test seem to be better than the mononucleosis spot itself. For their monospot tests Wolf et al used the Monolatex test and they quote low sensitivities and specificities from 63% to 84% and 84% to 100%. However in an evaluation of 12 Commercial Tests for the detection of Epstein-Barr Virus-Specific and Heterophile Antibodies, Bruu et al found, for the purpose of detecting heterophile antibodies, that Clearview yielded the best results, with sensitivity and a specificity of 95 and 100% respectively (Bruu et al. 2000). They also comment that a result may take up to 48 hours, whilst in our institution the monospot
test is performed on request, taking no more time than a full blood count and differential.

From 1908 a relative lymphocytosis has been recognized as a prerequisite for diagnosis and in 1952 Hoagland and Bender recommended that mononucleosis be diagnosed only when both characteristic blood smears and heterophile antibodies reactions were observed. Furthermore, Hoagland warned that disregarding either the heterophile agglutination test or the characteristic blood picture, as prerequisites for diagnosis, would perpetuate considerable confusion and error (Hoagland 1960a).
1.4.4 Clinical applicability of the study

Sore throat is a very common presenting complaint to medical practitioners, with over 40 million visits by adults each year in the United States (Komaroff et al. 1986). In a series of 500 patients with confirmed infectious mononucleosis, at least 98% presented with a sore throat, lymph node enlargement, fever, and tonsillar enlargement and as such it can be difficult to distinguish clinically from acute bacterial tonsillitis. The importance in making this distinction lies not only in the different disease progression and management, but also with regard to the potential life threatening complications of infectious mononucleosis. Anecdotally, the pharyngeal symptoms associated with infectious mononucleosis are more severe than with bacterial tonsillitis. This is reflected in a longer length of stay in hospital, with IM having near twice the mean in patient stay as bacterial tonsillitis (Hospital Episode Statistics 2011). As with bacterial tonsillitis, infectious mononucleosis may be associated with peritonsillar abscess, with various articles reporting rates of peritonsillar abscess between 0.2 - 23% (Johnsen 1981, Johnsen et al. 1984, Arkkila et al. 1998, Ebell 2004). Although recurrent infectious mononucleosis is not common (Paterson and Pinniger 1955, McSherry 1982), it is associated with prolonged symptoms, with up to 9% developing chronic fatigue syndrome at 6 months (White et al. 1998). In general infectious mononucleosis is treated conservatively with supportive measure but there are a number of studies in the literature advocating the use of Metronidazole, with objective improvement in the clinical symptoms of tonsillar hypertrophy and a reduction in the duration of pyrexia (Davidson et al. 1982, Spelman and Newton-John 1982, Dalmau et al. 1990). Intubation and or surgery may be indicated in the acute phase, in patients with upper airway obstruction whose condition does not improve after intensive medical management. Those patients with severe infectious mononucleosis requiring steroid treatment were
also found to be more likely to require a tonsillectomy subsequently for recurrent bacterial tonsillitis (Stevenson et al. 1992, Chan and Dawes 2001). Life threatening complications can occur in up to 5% of cases, and include upper airway obstruction, splenic rupture, neurological complications, pericarditis and myocarditis (Daniels et al. 1979).

The study by Wolf et al recommended that the L/WCC should be used as a screening tool and our results concur that a high L/WCC is a good indicator of infectious mononucleosis. However, if we were to follow the suggested algorithm and not perform a monospot test on patients with a L/WCC less than 0.35, one in seven of the patients with infectious mononucleosis would be missed leading to mismanagement and potentially increase morbidity from the disease. We therefore propose that the L/WCC ratio should be used in conjunction with the Hoagland criteria.
CHAPTER 2

A longer stay for the kissing disease: epidemiology of bacterial tonsillitis and infectious mononucleosis over a 20-year period

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Conception and Design: PL, JEF
Analysis and Interpretation: JS
Data Collection: PL
Writing the article: PL
Critical Revision of article: JEF
ABSTRACT

Introduction
Anecdotally Infectious Mononucleosis is a more severe infection than Bacterial Tonsillitis, requiring a longer hospital stay. There is little in the literature comparing the epidemiology of the two. The aim of our study was to compare the epidemiology of Bacterial tonsillitis and Infectious Mononucleosis and in particular the difference in length of in hospital stay between the two.

Methodology
The hospital in-patient enquiry system was used to analyse patients admitted with Infectious Mononucleosis and Bacterial Tonsillitis, between 1990 and 2009 inclusive.

Results
There were a total of 3435 cases over the 20 years, 3064 were Bacterial Tonsillitis and 371 were infectious mononucleosis. The mean length of stay for Bacterial Tonsillitis was 3.22 days and Infectious Mononucleosis was 4.37. The median length of stay’s were tested using the Mann-Whitney U nonparametric test and were found to be significantly different (p<0.001)

Conclusion
Infectious mononucleosis requires a significantly longer stay in hospital than Bacterial Tonsillitis.
2.1 Introduction

Epstein-Barr virus (EBV) is one of the most common viral infections in human (Masucci and Ernberg 1994). In childhood, the disease is usually subclinical and early infection is associated with poor hygiene and over crowding. Most of the population in the lower socioeconomic groups will have acquired immunity by adolescence (Schuster and Kreth 1992).

EBV infection of adolescents or adults results in Infectious Mononucleosis in up to 70% of cases and can prove severe (Tattevin et al. 2006). Improved housing and decreasing family sizes may have resulted in a change in the epidemiology of EBV and therefore infectious mononucleosis. Acquisition of EBV is often delayed in more affluent social classes resulting in increased hospital admission (Crowcroft et al. 1998). A recent increased rate of hospital admission has been documented (Morris and Edmunds 2002, Tattevin et al. 2006).

Anecdotally Infectious Mononucleosis is reported to be a more severe infection than bacterial tonsillitis (BT) thus requiring a longer stay in hospital, however there is no evidence in the literature to support this. The aim of our study was to compare the duration of hospital admission for patients admitted with severe bacterial tonsillitis and Infectious Mononucleosis.
2.1 Methods

A 20-year retrospective analysis was undertaken, between the years 1990-2009 inclusive. We compared hospital admissions for BT and IM in the University Hospital, Limerick, Ireland. Epidemiological information was acquired through the Hospital in Patient Enquiry System. Those coded for emergency admission with infectious mononucleosis and bacterial tonsillitis under the otolaryngology department were included. The indications for admission were inability to take oral antibiotics, airway management, and requirements for intravenous rehydration or lack of response to oral therapy. Bacterial Tonsillitis is often diagnosed clinically, and in accordance to the Hoagland criteria if Infectious Mononucleosis is suspected, a patient with fever, pharyngitis, lymphadenopathy, and lymphocytosis, can have the diagnosis confirmed by positive serological test (Hoagland 1975, Lennon et al. 2010). Those under 15 were excluded as they were primarily treated by the paediatric service. This data was compared to patients admitted with acute tonsillitis. Those with peritonsillar abscesses were excluded. Incidence and length of stay were evaluated, was age and sex of the patients. Data was analysed with Mann-Whitney U nonparametric, Fishers exact and chi-squared, and nonparametric Kruskal-Wallis tests by the Statistical Consulting Unit at the Graduate Entry Medical School, University of Limerick.

Incidence rates were calculated by using population data by county, provided by the Central Statics Office, Ireland. These were generated from census data over the 20-year period (Central Statistics Office, Ireland 2011). Counties in the Mid-Western area that are covered by the University Hospital include Limerick, Clare and North Tipperary.

2.3 Results
There were a total of 3435 cases over the 20 years, 3064 were Bacterial Tonsillitis and 371 were Infectious Mononucleosis. The incidence rates are for the total mid-west population. There was 1.6 cases of Infectious Mononucleosis per 100,000 in 1990 requiring hospital admission and 5.5/100,000 in 2009. The highest incidence of Infectious Mononucleosis occurred in 2000 with 7.8/100.000. There were 27/100,000 cases of Bacterial Tonsillitis in 1990 and 45/100000 in 2009. The peak year for Bacterial Tonsillitis was 2001 with 64/100,000 (Fig 3).

![Figure 3: Prevalence rates of Bacterial Tonsillitis and Infectious Mononucleosis 1990-2009](image)

The mean age for Bacterial Tonsillitis was 25.7 yrs, median age 22 yrs, SD 11.06 and range 15-87 yrs. The mean age for Infectious Mononucleosis was 20.0 yrs, median age 18 yrs, SD 6.32 and range 15-70 yrs. The distributions of age for both conditions were positively skewed. The median ages were tested between the conditions using the Mann-Whitney U nonparametric test and were found to be significantly different (p<0.001), the average age of those with Infectious Mononucleosis were significantly lower than those with Bacterial Tonsillitis (Fig 4, Fig 5).
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Figure 4: Age distribution of patients diagnosed with BT

Figure 5: Age distribution of patients diagnosed with IM
Numbers of each gender per disease were summarised and tested for difference between conditions and this was not significant (p=0.098) using Fishers exact test. However this is almost significant and it should be noted that there was a larger percentage of females in the bacterial tonsillitis group and a larger percentage of males in the Infectious Mononucleosis group.

Length of stay was also tested between diseases and the distribution was positively skewed again. The mean length of stay for Bacterial Tonsillitis was 3.22, median 3, SD 1.54 and range 1-19. The mean length of stay for Infectious Mononucleosis was 4.37, median 4, SD 2.37 and range 1-15.

Figure 6: Length of stay- BT
The median length of stay were tested between the conditions using the Mann-Whitney U nonparametric test and were found to be significantly different (p<0.001) with the median length of stay of those with Bacterial Tonsillitis being significantly lower than those with Infectious Mononucleosis, (Fig 6, Fig7, Fig8).
Mean length of stay was significantly different between ages ($p<0.001$). Means tended to increase with age. Again mean length of stay tended to be higher for those with Infectious Mononucleosis than Bacterial Tonsillitis (Fig 9)
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Figure 9: Length of stay per age by IM and BT
2.4 Discussion

The incidence of Infectious Mononucleosis did increase during the 20-year period from 1.6 to 5.5/100,000. The incidence peaked in the late 1990s and early 2000s. The mean incidence was 5.4/100,000. Fig 10. This compares to an admission rate of 4.2/100,000 in England between 1998 and 2005 (Ramagopalan et al. 2011). A rise in incidence of hospital admissions for Infectious Mononucleosis has also been noted in England and Wales from 2.6-4.8/100,000 from 1989-1998, (Morris and Edmunds 2002).

Figure 10: IM rate per 100,000 population (current study versus quoted rate in England)

A decrease in GP visits, especially in the young, along with the increase in hospital admission led Morris et al to conclude that the most likely explanation for the patterns they observed would be that falling childhood infection rates have resulted in an increase in the number of teenagers who are susceptible to a severe primary infection.
EBV infection in childhood is associated with low socioeconomic status (Crowcroft et al. 1998), so the economic boom Ireland experienced from the mid 1990s to the late 2000s may have resulted in a change in the epidemiology of EBV and therefore infectious mononucleosis (FitzGerarld 1999, GDP per capita in Purchasing Power Standards (PPS) (EU-27 = 100) 2011). However it is likely that both our report and that of Morris et al have too short a lag phase to predict a continuing trend in the rise in incidence of Infectious Mononucleosis.

We found a significantly younger age for those admitted with Infectious Mononucleosis than Bacterial Tonsillitis. In developed countries the highest incidence is in the 15-to-25 year-old age group (Evans and Kaslow 1997). Therefore a mean age of 20 for Infectious Mononucleosis is in line with other epidemiological reports. A mean age of 18.3 was found in Wisconsin in 1961 (Evans 1961), and 19.3 years in Norway in 1978 (Munoz et al. 1978, Evans and Kaslow 1997). A slightly younger mean age was found for females than males in one paper (female 17·0 years, males 19·5 years) which was explained in that paper by earlier maturity of females(Odegaard 1967). Infectious Mononucleosis is more likely to occur earlier in developing countries(Carvalho et al. 1973), whilst Infectious Mononucleosis is rare in the elderly(Axelrod and Finestone 1990). A review of the literature between 1968-1987 found just 29 cases in patients over 60 (Schmader et al. 1989). There was a single patient over 60 with Infectious Mononucleosis in our cohort of 371. The mean age of patients admitted with Bacterial Tonsillitis was 25.7 years. Although children from the age of 5-15 years old are most likely to be diagnosed with Bacterial Tonsillitis (Clinical Knowledge Summaries 2011), when an adult is admitted they tend to be older than patients with Infectious Mononucleosis. Studies with mean ages of 27.3 years (Bhattacharyya and Kepnes
2002) and 27.7 years (Gallegos et al. 1995) have been published whilst a significantly lower age of 23 for Infectious Mononucleosis compared with 27 was also noted in a study (Wolf et al. 2007).

Although not significant, we noted larger percentage of females in the Bacterial Tonsillitis group and a larger percentage of males in the Infectious Mononucleosis group. Another recent paper found a larger percentage of females in the Infectious Mononucleosis group in their study. This too was not significant (Mahmud et al. 2011). Overall Infectious Mononucleosis seems to occur equally in both sexes (Evans and Kaslow 1997).

The average length of stay was found to be significantly longer for IM (mean 4.37, median 4 days) than BT (mean 3.22, median 3 days). For Infectious Mononucleosis this again is comparative to the literature with a study from 2005 having a mean length of stay of 4.6 days (Thompson et al. 2005). However in one paper the average length of stay for Infectious Mononucleosis was more than double of what we found at 9.2 days in Hungary (Almasi et al. 2001). Raw data from the NHS shows a mean length of stay of 2.2 days for Infectious Mononucleosis and 1 day for acute tonsillitis in 2009-10 (Hospital Episode Statistics 2011). This is not likely to represent severe Bacterial Tonsillitis, however there is little in the literature regarding length of stay for Bacterial Tonsillitis. One report from Maine in 1975 gave a mean length of stay at 3.28 days (Wennberg et al. 1975). Infectious Mononucleosis in patients over 40 often present with atypical signs and symptoms and can therefore have a longer hospital stay (Halevy and Ash 1988).
A longer length of stay may be due to fact that Infectious Mononucleosis is generally treated supportively, as it is a viral infection. Acyclovir has been shown to be ineffective in treating the symptoms of Infectious Mononucleosis (Torre and Tambini 1999) and although steroids have only been shown to of benefit in cases of upper airway obstruction they do not decrease the length of stay (Thompson et al. 2005). As a bacterial disease, tonsillitis is most commonly limited to the pharynx, whilst Infectious Mononucleosis is a systemic disease. Tonsils have shown to be more susceptible to bacterial infection with Infectious Mononucleosis (Stenfors et al. 2001, Stenfors et al. 2003) in particular anaerobic bacteria (Brook 2005). This has led to some treating Infectious Mononucleosis with Metronidazole, and several studies have demonstrated faster acute and long-term recovery in Infectious Mononucleosis with metronidazole (Hedstrom et al. 1978, Hedstrom 1980, Davidson et al. 1982, Spelman and Newton-John 1982, Dalmau et al. 1990). Infectious Mononucleosis can also be difficult to diagnose (Lennon et al. 2010), which may lead to a delay in treatment and is also associated with more severe complications than Bacterial Tonsillitis.

Physicians and hospital managers are increasingly under pressure to monitor and improve economic performance. Length of stay data is increasingly used to monitor economic performance, (Rapoport et al. 2003). Increased length of stay is associated with higher cost per patient (Stock and McDermott 2011). Expected length of stay is used health indicator of efficiency in many countries (Information 2005). In this environment therefore it important to note the significant difference we found between the length of stay of Infectious Mononucleosis and Bacterial Tonsillitis.
The strengths of the paper lie in the fact that it a 20 year study from a single institution comparing large numbers of commonly treated diseases. The data is from a single Hospital in Patient Enquiry System and the Consultant Biostatistician at a University Medical School carried out the statistical analysis. The weaknesses include that readmission were not identified in the study. Also the fact that treatment of both Infectious Mononucleosis and Bacterial Tonsillitis would have been carried out by a large number of different physicians over the 20-year period and allow that different treatment regimes may have been in place throughout the years. Therefore we must rely on the accuracy of the Hospital in Patient Enquiry System.
2.5 Conclusions

This is a large epidemiological study with a long time line. There are not many such studies on Infectious Mononucleosis in the recent literature and none comparing Infectious Mononucleosis to Bacterial Tonsillitis. Our results concur with many of the studies on the incidence, age and sex distribution of Infectious Mononucleosis and Bacterial Tonsillitis and a trend to an increasing incidence of Infectious Mononucleosis has also been noted. We have however for the first time demonstrated that Infectious Mononucleosis is indeed a more severe infection, with a significantly longer stay in hospital.
2.6 Summary

- Infectious Mononucleosis is a differential for Bacterial Tonsillitis
- Anecdotally Infectious Mononucleosis is a more severe infection than Bacterial Tonsillitis
- There is a trend of increasing incidence of Infectious Mononucleosis
- Severe Infectious Mononucleosis occurs in a younger age than Bacterial Tonsillitis
- Infectious Mononucleosis requires a longer stay in hospital
CHAPTER 3

Effect of metronidazole versus standard care on length of stay of patients admitted with severe infectious mononucleosis: a randomized controlled trial

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Clinical Microbiology and Infection: PMID 24329850

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Writing the article: PL

Data Collection: PL

Critical Revision of article: PL, JPON, JEF
ABSTRACT

Metronidazole may be of use in the treatment of infectious mononucleosis (IM). Our aim is to show that metronidazole shortens hospital stay for patients with severe IM. A single-centre randomized controlled trial was undertaken in patients admitted with severe IM, who were with a similar group treated by the standard care. Patients were blinded to which treatment arm they were in. Forty-two of these patients were enrolled in the trial. The primary endpoint was the difference in length of stay. This was significantly less in the metronidazole group (3.67 days v 4.67) (p 0.032). This study demonstrates that metronidazole has a role to play in severe infectious mononucleosis.
3.1 Introduction

Epstein Barr Virus (EBV) is one of the most common viral infections in human beings (Masucci and Ernberg 1994). In childhood, the disease is usually subclinical, and most of the population will have acquired immunity by adolescence (Schuster and Kreth 1992). However infection of adolescents or adults results in Infectious Mononucleosis (IM) in 30 to 70% of cases and can prove severe (Tattevin et al. 2006), with these patients having a significantly longer hospital stay than those admitted with a bacterial tonsillitis. Metronidazole is an antibiotic medication used primarily in infections caused by anaerobic bacteria and protozoa. There is some evidence that this antibiotic may also be efficacious in the treatment of IM (Hedstrom et al. 1978). A possible mechanism of its action in infectious mononucleosis is through suppression of the oral anaerobic flora (Brook and de Leyva 1994, Kopec et al. 1997, Brook 2005). Previous studies have demonstrated a more rapid regression of symptoms in those taking metronidazole (Hedstrom et al. 1978, Davidson et al. 1979, Hedstrom 1980, Davidson et al. 1982, Dalmau et al. 1990), however a single study showed no difference in those taking metronidazole (Spelman and Newton-John 1982). Therefore there is ongoing controversy and the treatment of IM with metronidazole has not gained widespread acceptance. The hypothesis of our study is that metronidazole does shorten hospital stay and our primary objective is to demonstrate this by undertaking a randomized controlled trial in adults admitted with severe IM and compare them to a similar group treated by the standard of care.
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3.2 Methods

3.2.1 Trial design, inclusion and exclusion criteria

The trial was a single centre prospective randomized controlled trial. Each patient was randomized to the control or intervention arm of the trial on a one to one basis. Both males and female patients diagnosed with IM were eligible for inclusion in the trial if they were 15 or over and admitted under the care of the Otolaryngology team at the University Hospital, Limerick, a tertiary referral centre for the mid-western region of Ireland. Patients were diagnosed with IM if they fulfilled the Hoagland criteria; that is presenting with a triad of fever, pharyngitis, and lymphadenopathy, with at least a 50% lymphocytosis, and confirmed by positive serological test (Hoagland 1975, Ebell 2004). Patients were excluded if they were allergic to penicillin and therefore would get an alternative to Benzylpenicillin, as is the standard of care. Pregnant patients and those with airway emergencies were also excluded. Patients diagnosed with CMV mononucleosis or other mononucleosis like infections were also excluded. A Cochrane review (Candy and Hotopf 2006) found that there was no evidence for the routine treatment of IM with steroids, however it was felt that this may be a confounder, therefore initially these patients were also to be excluded. However another review recommended steroids for all patients presenting with sore throats (Hayward et al. 2012), and although Emergency department staff were educated about the study, a number of patients who were given steroids in the Emergency department were included in the study. Steroids were not continued once the patients were admitted and ancillary analysis was performed on these patients.
3.2.2 Metronidazole dosing

Metronidazole is a well established nitroimidazole antibiotic. A standard dose of 500mg TDS intravenously was given at regular interval at the same time as the standard of care antibiotic, Benzylpenicillin. Benzylpenicillin is given to patients with IM who are not penicillin allergic to prevent a bacterial superinfection. It is part of the standard of care, including supportive treatment with intravenous hydration, antipyretics and analgesics, which all patients received. No placebo was given as nurses on the hospital ward gave both the antibiotics at the same time.

3.2.3 Outcomes

The primary outcome measured was the difference in length of stay in days in the hospital. Length of stay (LOS) was acquired from the case report form and verified by the Hospital In Patient Enquiry (HIPE) system. Patients were discharged when the consultant in charge of their care felt that they had made a sufficient recovery and were unlikely to represent or require further inpatient care. Secondary outcomes included reduction in inflammatory markers, (white cell count, WCC) and duration of high temperature (Over 38 degrees Celsius).

3.2.4 Sample size calculation

The specified elements of the sample size calculation that we would want to include in our trial report are a significance level of 5%, power of 80%, a baseline mean hospital stay of 4.37 days, (using data from a recently published study on the same population (Lennon et al. 2013b)), and a decrease in hospital stay by 25%. The target total was 48 patients. Interim analysis was not carried out.
3.2.5 Randomisation

Randomisation was carried out by simple randomization, and allocation was concealed from the patient. The first author carried out enrolment, randomization and allocation concealment. Patients were blinded to their treated arm. Both received multiple injections and nurses were asked not to inform patients of which arm the patient was in.

3.2.6 Statistical analysis

Statistical analysis on the difference in length of stay between the arms of the trial will be calculated, along with 95% confidence intervals, using the statistical program SPSS. Subgroup analysis was carried out on those who received steroids. CONSORT (CONsolidated Standards of Reporting Trials) guidelines were followed during the trial (Moher et al. 2010).

3.2.7 Ethics

Ethical permission was sought and granted by the Mid-Western Regional Ethics Research committee and all patients signed an informed consent prior to being enrolled in the trial.
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3.3 Results

3.3.1 Recruitment

Fifty-one patients were admitted under the Ear Nose and Throat service with Infectious Mononucleosis during the trial period. Forty-two of these patients were enrolled in the trial. Eight patients were excluded, 5 as they were allergic to penicillin, 2 due to being under the age of 15, and one patient with an airway emergency. A single patient refused to partake in the trial. Age eligible patient were recruited between the 5th January 2010 and the 11th April 2012. Patients were followed as in-patients and therefore there was no loss to follow up. Three patients were started on Metronidazole who were initially in the control group, but failed to show any sign of improvement. These patients were analysed in their original group (Fig 11). The trial was stopped when sufficient numbers were recruited to allow statistical analysis.

![Patient flow diagram](Figure 11: Patient flow (CONSORT) diagram)
3.3.2 Patient Characteristics

Both groups had a similar age profile, with a mean age of 18.26 (Fig 12) and 18.56 (Fig 13) in the intervention and control arm respectively.

![Age on admission - Metronidazole group](image)

Figure 12: Age on admission-metronidazole group

There was a larger amount of females in the standard of care group but this was not found to be significant (Chi-square, p=0.352). Both groups were found to be symptomatic for a similar period prior to admission (6.5 and 6.3 mean days) and had attended their primary care physician who had prescribed antibiotics for on average 3.25 and 3 days prior to admission to hospital.
Fifteen in the treatment arm and 17 in the control arm had received antibiotics prior to admission. No significant difference in these baseline values was found between the two groups (Table 7).

Table 7: Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Metronidazole</th>
<th>Standard of Care</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean, SD)</td>
<td>18.26 (2.2)</td>
<td>18.56 (2.03)</td>
<td>0.643</td>
</tr>
<tr>
<td>Age (Range)</td>
<td>15-23 years</td>
<td>15-22 years</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Days Symptomatic prior to admission (mean)</td>
<td>6.5</td>
<td>6.3</td>
<td>0.872</td>
</tr>
<tr>
<td>Attended GP prior to admission</td>
<td>15</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>
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| Days receiving antibiotic prior to admission (mean) | 3.25 | 3 | 0.649 |

3.3.3 Outcomes

The primary endpoint in the study was the difference in length of stay between the two groups. This was significantly less in the Metronidazole group with a mean of 3.67, compared to a mean of 4.67 in the standard of care group (p=0.032). There were small differences in the amount of days the patients remained febrile and the rate of decrease in the WCC, however neither of these were significant. We also found no significant difference in the LOS between those that had received or not received steroids, and no significant difference between the sexes or those that presented less than 5 days after the symptoms began or those that presented later than this (Table 8).

Table 8: Results of the RCT
No adverse effects from Metronidazole were reported and there were no complications due to the disease process in either group.
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3.4 Discussion

Infectious Mononucleosis caused by Epstein Barr virus was in its infancy as a recognisable disease process when Hedström et al suggested that the pharyngotonsillitis elements of the illness were reminiscent of an anaerobic infection. A number of studies were therefore undertaken to examine the possible role of Metronidazole in the treatment of IM. The majority of these studies support the contention that Metronidazole does indeed have a beneficial action in those with severe IM (Hedstrom et al. 1978, Hedstrom 1980, Davidson et al. 1982, Dalmau et al. 1990), with a single paper showing no significant effect (Spelman and Newton-John 1982). The results of our study here concur with the majority of those previously undertaken that Metronidazole shortens the duration of severe IM.

3.4.1 Limitations of the study

The limitations of this study include its small size, and therefore are confidence intervals are rather wide, however they do not cross 0. The trial is similar in size to the larger of the previously published studies on the same subject (Hedstrom 1980, Spelman and Newton-John 1982). Due to their small size and that the methods differ between them substantially, meta-analysis of these studies may be misleading (Walker et al. 2008). The lack of publications with ‘negative’ results for Metronidazole treatment may be due to publication bias towards those with ‘positive’ results. From a recently published study on the epidemiology of the same population (Lennon et al. 2013b), a mean of 18.5 patients a year were admitted over the previous 20 years at this institution. Therefore recruitment of 42 patients out of a possible 51 patients in 28 months was deemed satisfactory.
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Further weaknesses included the lack of a placebo. The trial was carried out within a clinical setting with no added funding. Medical and nursing staffs were educated about the study and efforts were made to conceal the treatment allocation from patients. There was also a very small trial team, with the primary author carrying out most of the recruitment, allocation, randomization, data gathering and inputting, as well as the statistical analysis, thus leading to the risk of multiple biases, including reporting bias and multiplicity. Randomization was also done by simple randomization where, permuted blocked may have been a better choice initially. However baseline values in both groups are similar.

3.4.2 Synopsis of key findings

Length of stay was used as our primary outcome as it could be reliably measured and verified by hospital records, and compared against other studies. The patients’ fitness for discharge was used as our main endpoint as an overall assessment of their wellbeing, as no validated scoring system for IM exists to our knowledge. We also examined whether Metronidazole had an effect on the persistent high temperatures in these patients. We used antipyretics in all the patients, as this was the standard of care and a stipulation of our ethical approval. In some of the older papers (Hedstrom 1980), antipyretics were avoided to fully assess the effect of Metronidazole. Whilst in hospital patients received regular antipyretics and few patients had persistent temperatures, with no significant difference seen between the two groups. We also did not see a significant difference between the groups in the rate at which their WCC fell. However it has been noted that in treating infectious diseases the “pursuit of the holy grail of laboratory
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markers continues. The clinical constellation of the context and history of illness, findings on examination and simple laboratory tests together trump any individual test” (Long 2006).

3.4.3 External validity

The results of this trial can be applied to a small but noteworthy cohort of patients. That is, those with severe IM that require admission to hospital. A large amount of patients that develop IM are likely to be treated in the community and a study on the benefits of Metronidazole on these patients would also be interesting.

3.4.4 Comparison to other studies

The results of our trial concur with four of the previously published reports on the use of Metronidazole in IM. We noted a mean of a day shorter in hospital between our two groups. Hedström et al noted a ‘significantly shorter period of tonsillitis’ (Hedstrom et al. 1978) with most of their patients having no signs of tonsillitis after 3 days in those treated with metronidazole compared to 4-7 days in the controls. In a follow up study they again noted that those treated with Metronidazole were significantly better on the third day (Hedstrom 1980). A further two papers (Davidson et al. 1982, Dalmau et al. 1990), one with a paediatric population, demonstrated a rapid regression of symptoms in uncontrolled small groups. In our study, three patients were started on Metronidazole that were originally in the control group, as they failed to improve or had deteriorated. The three patients subsequently made a rapid recovery. A paper by Spelman et al (Spelman and Newton-John 1982) also demonstrated that patients were greatly improved by the fourth day but they found no significant difference between the treatment and control group (Table 3).
Table 9: Literature review—Previously published reports on the use of Metronidazole in IM.

<table>
<thead>
<tr>
<th>Name</th>
<th>Year</th>
<th>Number of patients</th>
<th>Result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intervention</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Hedström(Hedstrom et al. 1978)</td>
<td>1978</td>
<td>16</td>
<td>10</td>
<td>‘Significantly shorter’ penicillin</td>
</tr>
<tr>
<td>Hedström(Hedstrom 1980)</td>
<td>1980</td>
<td>21</td>
<td>19</td>
<td>significantly better antipyretics given</td>
</tr>
<tr>
<td>Davidson(Davidson et al. 1982)</td>
<td>1982</td>
<td>5</td>
<td>0</td>
<td>All improved shortly after treatment Paediatric population</td>
</tr>
<tr>
<td>Spelman and Newton-John 1982</td>
<td>1982</td>
<td>20</td>
<td>20</td>
<td>No significant difference</td>
</tr>
<tr>
<td>Dalmu(Dalmau et al. 1990)</td>
<td>1990</td>
<td>4</td>
<td>0</td>
<td>Rapid clinical improvement</td>
</tr>
</tbody>
</table>
A much greater number of anaerobic bacteria have been found on the surface of tonsils during an infection with IM than when the patient has recovered from the disease (Brook and de Leyva 1994). It has also been shown that EBV exerts a transient suppression of immunoglobulin-coating of bacteria harboured on the tonsillar surfaces, with consequent abundant bacterial attachment to the epithelial cells and massive bacterial colonization on the palatine tonsils and penetration into the epithelial cells (Stenfors and Raisanen 1996, Stenfors et al. 2001). The role of anaerobic bacteria in the pharyngitis associated with IM is difficult to elucidate because these organisms are part of the normal oropharyngeal flora. However it has been postulated that Metronidazole may help to hasten recovery in these patients by suppressing of the oral anaerobic flora that might contribute to the inflammatory process induced by the Epstein–Barr virus (Brook 2005).
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3.5 Conclusions

Our study has again demonstrated that Metronidazole has a role to play in severe Infectious Mononucleosis. We were able to demonstrate that patients randomised to metronidazole had a significantly shorter hospital stay than those who were treated by the standard of care. More trials on a larger scale and possibly in an outpatient setting are required.
CHAPTER 4

Infectious Mononucleosis

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Writing the article: PL

Critical Revision of article: MC, JEF
THE BOTTOM LINE

- Infectious mononucleosis is a clinical diagnosis, caused by Epstein-Barr virus in 90% of cases, although in some patients (pregnancy, high risk HIV population) further investigations are warranted

- Treatment should be supportive, with steroids given only in cases of airway compromise

- Treatment with antiviral agents has yet to be shown to be of benefit

- Patients wanting to return to contact sports before one month should undergo abdominal ultrasonography to rule out splenomegaly

- Splenic rupture should be considered with any abdominal pain in infectious mononucleosis
4.1 Introduction

Infectious mononucleosis is commonly seen in both the community and the hospital setting. Patients usually present with a sore throat and often presume that an antibiotic is required. It is therefore important to dispel the many myths relating to the condition with appropriate patient education. Knowledge of the clinical course of the disease, as well as potential complications, is paramount. In an information age, difficult questions may arise for a general practitioner, emergency doctor, or trainee in ear, nose, and throat medicine. The aim of this review is to assist those who encounter infectious mononucleosis in the adolescent and adult population
4.2 Sources and selection criteria

We performed an electronic search through Medline, Scopus, Google Scholar, the Cochrane Database of Systematic Reviews, and the Cochrane central register of controlled trials using the search terms “infectious mononucleosis”, “glandular fever”, “Epstein-Barr virus”, “corticosteroids”, and “acyclovir”. The search was limited to articles in English. We excluded studies carried out primarily on children. Priority was given to data from meta-analyses, reviews, and randomised controlled trials. Research on infectious mononucleosis was also given priority over articles exclusively relating to Epstein-Barr virus. We also examined guidelines produced by the US Centre for Disease Control and Prevention and the UK National Institute for Health and Care Excellence, as well as clinical trials registries of the United States, United Kingdom, and European Union (Clinical trials for Infectious Mononucleosis, EU Clinical Trials Register 2014, ClinicalTrials.gov, U.S. National Institutes of Health 2014, Current controlled trials, International Standard Randomised Controlled Trial Number Register 2104).
4.3 What is infectious mononucleosis and what causes it?

It would be most accurate to consider infectious mononucleosis as a non-genetic syndrome, defined by the classic triad of fever, pharyngitis, and cervical lymphadenopathy, where lymphocytosis is also present. For many doctors the terms Epstein-Barr virus and infectious mononucleosis are synonymous. Epstein-Barr virus causes approximately 90% of the cases of infectious mononucleosis, with the remainder due largely to cytomegalovirus, human herpes virus 6, toxoplasmosis, HIV, and adenovirus (Henle et al. 1974, Hurt and Tammaro 2007). The World Health Organization’s ICD-10 (international classification of diseases, 10th revision) has four subheadings for infectious mononucleosis (or B27 in the manual (International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) 2010)): infectious mononucleosis associated with Epstein-Barr virus (B27.0), cytomegalovirus infectious mononucleosis (B27.1), other infectious mononucleosis (B27.8), and infectious mononucleosis unspecified (B27.9). To confuse things further the multiple synonyms for infectious mononucleosis (glandular fever, monocytic angina, Pfeiffer’s disease, Filatov’s disease, Drusenfieber, and even the kissing disease) are still included in ICD-9, which will be in use in the United States until 1 October 2015 (ICD-9-CM Diagnosis Code 075 2012).

The Epstein-Barr virus is a ubiquitous herpes virus, with more than 90% of the world’s population infected by adulthood (Henle et al. 1969). The virus is one of our most effective parasites (Maesween and Crawford 2003) and remains as a lifelong, latent infection, by integrating itself into the life cycle of healthy B lymphocytes (Thorley-Lawson et al. 1996, Babcock et al. 1998). There is persistent low grade replication and the virus is shed intermittently into pharyngeal secretions, particularly saliva, through which it is transmitted. These low titres of infectious virus account for the low to
moderate contagiousness (Niederman et al. 1976, Yao et al. 1985) of the disease and the apparent requirement of intimate contact for disease transmission (Andiman 2006). During an active infection the viral load may be increased, and therefore some precautions about contact should be mentioned (cough etiquette, hand hygiene, kissing, sharing food or utensils); however, as most of the population is positive for Epstein-Barr virus, special precautions against transmission are not necessary in most cases (Luzuriaga and Sullivan 2010). Childhood infection, which is usually subclinical, is associated with poor hygiene and over-crowding. In lower socioeconomic groups most of the population will have acquired immunity by adolescence (Schuster and Kreth 1992). After an incubation period of four to seven weeks (Hoagland 1964), EBV infection of adolescents or adults results in IM in up to 70% of cases (Tattevin et al. 2006). Most symptoms tend to resolve in two to four weeks, although approximately 20% of patients continue to mention a sore throat at one month (Rea et al. 2001). In one study, patients with severe infectious mononucleosis who were admitted to hospital for intravenous hydration required a significantly longer stay than those admitted with bacterial tonsillitis (Lennon et al. 2013b). Reactivation of Epstein-Barr virus may occur in immunocompromised patients (Ternak 2003) and, rarely, in immunocompetent patients, which may lead to Epstein-Barr virus associated lymphoproliferative conditions. These are a heterogeneous group of diseases that often need to be treated with chemotherapy (Tse and Kwong 2015). Diagnoses depend on the specific disease but are often associated with an increased viral load (Ok et al. 2015). Chronic active Epstein-Barr virus infection is a rare condition that is typified by severe, chronic, or recurrent infectious mononucleosis-like symptoms after a well-documented primary infection with Epstein-Barr virus in a previously healthy person (Macsween and
Crawford 2003). Chronic active Epstein-Barr virus infection is occasionally associated with the development of lymphoma (Kanegane et al. 2002).
4.4 How is it diagnosed?

Infectious mononucleosis may account for as little as 1% of patients who present with a sore throat to their doctor (Bisno 2001). Non-specific prodromal symptoms of fever, chills, and malaise may be seen in infectious mononucleosis. These symptoms may also be present in cases of viral pharyngitis, commonly caused by rhinovirus, adenovirus, and coronavirus. Whereas these viruses generally give rise to symptoms of a common cold (Bisno 2001), clinically infectious mononucleosis should be suspected in anyone who presents with fever, pharyngitis, and cervical lymphadenopathy (the classic triad of fever, pharyngitis, and cervical lymphadenopathy) (Pfeiffer 1889). Lymphadenopathy may be prominent in both the anterior and the posterior triangles of the neck, which distinguishes infectious mononucleosis from bacterial tonsillitis (where the lymphadenopathy is usually limited to the upper anterior cervical chain). These signs were found in 98% of patients with a diagnosis of infectious mononucleosis (Hoagland 1975). Other common physical signs include palatal petechiae (25-50%), splenomegaly (8%) (Rea et al. 2001), hepatomegaly (7%), and jaundice (6-8%) (Hoagland 1960a), with a transitory derangement of liver function tests (in particular increased aspartate aminotransferase and alanine aminotransferase levels, returning to normal after 20 days) seen in 80-90% of patients (Kofteridis et al. 2011). Anecdotally, a “whitewash” exudate on the tonsils may also help to distinguish infectious mononucleosis from the more speckled exudate of bacterial tonsillitis and the erythema of a viral pharyngitis that is void of exudate. In the primary care setting a clinical diagnosis alone may be sufficient to allow adequate management of a patient. However, should a definitive diagnosis be sought, the Hoagland criteria states that in patients presenting with clinically suspected infectious mononucleosis and at least a 50% lymphocytosis (10% atypical), the diagnosis should be confirmed by the heterophile
antibody (monospot) test (Hoagland 1975). Using a lower rate of lymphocytosis (Wolf et al. 2007, Biggs et al. 2013) has been shown to give a greater rate of false negative results (Table 10) (Lennon et al. 2010, Lennon et al. 2013a, Lennon et al. 2014b)

Table 10: Diagnostic test for Infectious Mononucleosis

<table>
<thead>
<tr>
<th>Tests</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious mononucleosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full blood count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LWCC &gt;50%+10% atypical lymphocytes</td>
<td>61</td>
<td>95</td>
<td>An increase in lymphocyte count tends to lead to a greater specificity but poorer sensitivity</td>
</tr>
<tr>
<td>LWCC &gt;38%</td>
<td>84</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Monospot</td>
<td>71-98</td>
<td>91-99</td>
<td>Results vary between different available commercial kits</td>
</tr>
<tr>
<td>Antibody to VCA or EBNA</td>
<td>97</td>
<td>94</td>
<td>May have replaced monospot as standard investigation in some countries</td>
</tr>
<tr>
<td>Bacterial tonsillitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antistreptolysin O titre</td>
<td></td>
<td></td>
<td>Peak value 3-8 weeks after infection, and thus not of value in acute setting</td>
</tr>
<tr>
<td>Throat swab</td>
<td>78</td>
<td>99</td>
<td>Delay of 2-3 days for result</td>
</tr>
<tr>
<td>Rapid streptococcal antigen test</td>
<td>84</td>
<td>94</td>
<td>Increased cost</td>
</tr>
<tr>
<td>LWCC/lymphocyte to white cell count ratio; VCA=antiviral capsid antigen; EBNA=Epstein-Barr virus nuclear antigen.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The heterophile test may also be falsely negative in up to 25% of adults in the first week of symptoms (Hoagland 1975, Hurt and Tammaro 2007). It is not always necessary to definitively diagnose a cause for infectious mononucleosis, but specific antibody tests are available. Patients are considered to have a primary Epstein-Barr virus infection if they are positive for antiviral capsid antigen IgM but do not have antibodies to Epstein-Barr virus nuclear antigen, which would suggest past infection. Levels of antiviral capsid antigen IgG will also increase in the acute phase and persist for the rest of the patient’s life, whereas the antiviral capsid antigen IgM will disappear after 4-6 weeks. The presence of antiviral capsid antigen IgG and Epstein-Barr virus nuclear antigen suggest past infection (Centers for Disease Control and Prevention (CDC) Epstein-Barr Virus and Infectious Mononucleosis, Laboratory Testing  2014). A recent review found that real time polymerase chain reaction and measurement of Epstein-Barr virus
viral load provide useful tools for the early diagnosis of infectious mononucleosis in cases with inconclusive serological results (Vouloumanou et al. 2012). In a small number of cases, where the patient is either pregnant or in a high risk group for HIV infection (injecting drug user or men who have sex with men), further testing for cytomegalovirus, HIV, and other possible causes for infectious mononucleosis should be undertaken (Sudarshi et al. 2008, Rayment et al. 2014). Figure 14 presents an algorithm for diagnosing infectious mononucleosis (Tsaparas et al. 2000).

Figure 14: Suggested algorithm for diagnosing Infectious Mononucleosis
4.5 How is it treated?

Infectious mononucleosis is a viral illness in most cases, and as such it can be treated with rest, hydration, analgesia, and antipyretics. Inadvertent treatment with ampicillin results in a fine macular rash in 90% of patients (Macsween and Crawford 2003). This should be distinguished from an urticarial rash seen in an allergic reaction. Studies have shown that symptoms experienced by patients are more severe for infectious mononucleosis than for bacterial tonsillitis (Lennon et al. 2013b). Antiviral treatment with acyclovir has been shown to significantly decrease the rate of oropharyngeal Epstein-Barr virus shedding (Torre and Tambini 1999). Some early trials found a significant positive overall effect in cases of infectious mononucleosis treated with acyclovir (Andersson et al. 1986) and that it was useful in severe cases, with airway compromise. However, a meta-analysis of five studies found no evidence to support its use in the acute setting: an improvement in oropharyngeal symptoms was observed in 25 out of 59 (42.4%) patients treated with acyclovir and in 18 out of 57 (31.6%) control patients (odd ratio 1.6, 95% confidence interval 0.7 to 3.6; P=0.23) (Torre and Tambini 1999). Other antiviral treatments such as valaciclovir and ganciclovir (Adams et al. 2006) have shown some promise in the treatment of severe infectious mononucleosis and its complications and immunocompromised people. Two trials are in progress (ClinicalTrials.gov, U.S. National Institutes of Health 2014), but at present the routine use of both drugs is not advocated (Rafailidis et al. 2010). Anaerobic antibacterial agents such as metronidazole have been suggested to hasten recovery in infectious mononucleosis by suppression of the oral anaerobic flora that contribute to the inflammatory process (Brook 2005). This finding was borne out in some clinical studies (Hedstrom et al. 1978, Davidson et al. 1979, Hedstrom 1980, Davidson et al. 1982, Marklund et al. 1984, Dalmau et al. 1990), with a recent randomised controlled
trial showing the beneficial effects of metronidazole in severe infectious mononucleosis by shortening hospital stays (Lennon et al. 2014a). Larger trials may be required before the use of metronidazole is routinely recommended.
4.6 Are steroids of use in the treatment of infectious mononucleosis?

Several early reports supported the use of corticosteroids in the treatment of infectious mononucleosis (Prout and Dalrymple 1966). Further trials showed these effects to be short lived, with no significant difference between the control and intervention arm (Tynell et al. 1996). A Cochrane review was therefore undertaken, which concluded that there was insufficient evidence and the trials were too few, heterogeneous, and of poor quality to recommend steroid treatment for symptom control in glandular fever (Candy and Hotopf 2006). Another more recent Cochrane review concluded that corticosteroids increased the likelihood of both resolution and improvement of pain in participants with sore throat (Hayward et al. 2012); however, this review excluded publications on patients with a diagnosis of infectious mononucleosis. Steroid treatment should be considered in cases of airway emergency, in an attempt to temporise or preclude the need for intubation or tracheotomy (McGowan et al. 1992). Despite these guidelines, the use of corticosteroids remains widespread on a day to day basis (Thompson et al. 2005). Several reports have mentioned the adverse effects of corticosteroid use in infectious mononucleosis, including cases of peritonsillar cellulitis, acute onset diabetes mellitus, and neurological sequelae (Candy and Hotopf 2006).
4.7 Does infectious mononucleosis lead to chronic fatigue syndrome?

Chronic fatigue syndrome is defined as severe fatigue and disabling musculoskeletal and cognitive symptoms without another explanation that lasts for at least six months and results in severe impairment in daily functioning (Fukuda et al. 1994). There has been much debate about the cause of this disorder. Some authors suggest that it is precipitated by an acute infection, such as infectious mononucleosis, as many patients relate the onset of their illness to an initial infection from which they never recovered (Wessely et al. 1995). Prospective studies have reported an incidence of chronic fatigue syndrome of 7.3-12% in adults six months after infectious mononucleosis (White et al. 2001, Moss-Morris et al. 2011). However, the relation between chronic fatigue syndrome and infectious mononucleosis is still questionable. A study of over 1300 patients diagnosed as having infectious mononucleosis by serology, found that although 10% of patients reported fatigue none fulfilled the criteria for chronic fatigue syndrome (Table 11) (Petersen et al. 2006).

Table 11: Infectious Mononucleosis and Chronic Fatigue Syndrome

<table>
<thead>
<tr>
<th>Study</th>
<th>No of participants</th>
<th>Age of cohort (years)</th>
<th>% of patients with a diagnosis of CFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>White 1998#4</td>
<td>104</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>Buchwald 2000#7</td>
<td>150</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Candy 2003#5</td>
<td>62</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Petersen 2005#1</td>
<td>1318</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Hickie 2006#3</td>
<td>88</td>
<td>16</td>
<td>7.3</td>
</tr>
<tr>
<td>Moss-Morris 2011#4</td>
<td>246</td>
<td>16</td>
<td>7.8</td>
</tr>
<tr>
<td>Katz 2009#1</td>
<td>301</td>
<td>12-13</td>
<td>13</td>
</tr>
</tbody>
</table>

The cause of chronic fatigue syndrome is likely to be multifactorial. A trial that compared activity with imposed bed rest in the management of infectious mononucleosis found that those patients who were allowed out of bed as soon as they...
felt able reported a quicker recovery (Candy et al. 2002) A brief intervention at the time of diagnosis of infectious mononucleosis to allay fears of a prolonged disease may help to prevent the development of chronic fatigue syndrome (Candy et al. 2004). A recent editorial commented that chronic fatigue syndrome is unlikely to be a consequence of Epstein-Barr virus but a heterogeneous family of disorders arising from a constellation of pathophysiological causes('Chronic fatigue syndrome: going viral?' 2010).
4.8 When is it safe to return to sports?

Splenomegaly, evident on ultrasonography if not on palpation, occurs in almost all cases of infectious mononucleosis, and the risk of splenic rupture has been well established (Dommerby et al. 1986). A considerable number of 15-21 year olds will have infectious mononucleosis every year (Lennon et al. 2013b) and many of this population will be involved in contact sports (Nieman 1994). Strenuous or contact sports (for example, football, gymnastics, rugby, hockey, lacrosse, wrestling, diving, and basketball) or activities associated with increased intra-abdominal pressure, such as weightlifting, may put athletes at most risk (Auwaerter 2004).

Although recommendations of when to return to sport range from three (Kinderknecht 2002), four (Hoagland and Henson 1957), eight, and even up to 24 weeks (Moolenaar et al. 1988), no clinical guidelines are specific to infectious mononucleosis. The incidence of splenic rupture is less than 1% (Rea et al. 2001) and most occur in the initial three weeks of infectious mononucleosis, although cases have been described much later (Putukian et al. 2008).

Cases of spontaneous splenic rupture have also been described in the literature and doctors should have a high index of suspicion when abdominal pain is reported in the setting of infectious mononucleosis (Raman et al. 2014). A recent study involved weekly ultrasound examinations until resolution of splenomegaly. A mean increase in splenic length of 33.6% was observed, with a peak in enlargement on average 12.3 days from the onset of clinical symptoms. Most cases of splenomegaly had resolved by 4-6 weeks and there was a predictable rate of splenic regression of approximately 1% each day after reaching peak enlargement (Hosey et al. 2008). Similar results were reported
in another paper, with normalisation of spleens at one month in 84% of participants (O'Connor et al. 2011). One study recommended that athletes wanting to return to contact sport at 3-4 weeks should have an ultrasound examination to ensure that the spleen had returned to normal size (O'Connor et al. 2011). A systematic review published in 2014 advocated individualised recommendations for athletes (Becker and Smith 2014), and future work in this area may concentrate on splenic volume to allow a more accurate assessment of splenomegaly and risk.
4.9 Is multiple sclerosis caused by infectious mononucleosis?

There is evidence that a history of infectious mononucleosis significantly increases the risk of multiple sclerosis (Thacker et al. 2006) and that this association is far stronger than with other common childhood infections or afflictions. A meta-analysis concluded that the risk of multiple sclerosis seems to be greatest in those who were infected with Epstein-Barr virus at a later age (incidence begins to increase in adolescence, peaks around age 25 to 30 years, and declines to nearly zero by age 60) (thus developing infectious mononucleosis), with moderate risk for those infected with Epstein-Barr virus in early childhood, and close to zero risk in those not infected (Thacker et al. 2006) (Fig 15).

![Figure 15: Incidence of multiple sclerosis by Epstein-Barr virus infection](image-url)
A more recent meta-analysis showed that Epstein-Barr virus is present in 100% of cases of multiple sclerosis and therefore it has been suggested that the virus is not only a risk factor but also a prerequisite of multiple sclerosis (Pakpoor et al. 2013). Whether the association between multiple sclerosis and Epstein-Barr virus demonstrates a causal relation is, however, strongly debated (Pakpoor et al. 2013).

Although controversial, if proponents of the infectious mononucleosis-multiple sclerosis theory are correct, a vaccine against Epstein-Barr virus in theory could eradicate multiple sclerosis. In the only phase II trial of an Epstein-Barr virus vaccine in humans, rates of infectious mononucleosis were reduced in adults who were seronegative for Epstein-Barr virus, but the vaccine did not affect the rate of Epstein-Barr virus infection (Cohen et al. 2013). The development of a vaccine is challenging for several reasons, not least the long period between primary infection with Epstein-Barr virus and the development of many Epstein-Barr virus related tumours or multiple sclerosis (Cohen et al. 2011). To add further to the controversy it has been suggested that in lieu of a vaccine, a smaller, but still substantial, number of cases of multiple sclerosis could be prevented by exposing children to Epstein-Barr virus infection before adolescence (Thacker et al. 2006).
4.10 Is there an increased risk of lymphoma or other cancers after IM?

The association of Epstein-Barr virus with malignancies such as Burkitt’s lymphoma (Gromminger et al. 2012) in children and nasopharyngeal carcinoma (Song and Yang 2013) are well established. This review, however, focuses on patients presenting with infectious mononucleosis and it can be difficult to differentiate studies on Epstein-Barr virus and infectious mononucleosis about the risk of future malignancies. Two large Scandinavian cohort studies found a 2.55 to 2.83 times increased risk of Hodgkin’s lymphoma in patients with a diagnosis of infectious mononucleosis by heterophile antibody tests (Rosdahl et al. 1974, Hjalgrim et al. 2007). The results were similar in a recent British record linkage paper, which found a 3.44 risk ratio of Hodgkin’s lymphoma in the infectious mononucleosis cohort (Goldacre et al. 2009).

A review on Epstein-Barr virus related malignancies from 2014 commented that Hodgkin’s lymphoma is the only Epstein-Barr virus related malignancy, other than nasopharyngeal carcinoma, for which there is a body of evidence accumulated over time that establishes a strong association (Coghill and Hildesheim 2014). For other malignancies, a large prospective study found no clear association between a history of clinical infectious mononucleosis and risk of invasive breast cancer (Massa et al. 2012), and one of the cohort studies found that lung cancer was significantly less likely in the cohort with infectious mononucleosis (Hjalgrim et al. 2000).
4.11 Can infectious mononucleosis cause any complications?

Infectious mononucleosis in most cases resolves over a period of weeks, but may occasionally be exacerbated by a wide variety of complications. Neurological disorders may occur in 1-5% of patients (Jenson 2000). Theses include encephalitis, meningoencephalitis, seizures, optic neuritis, sudden sensorineural hearing loss, idiopathic facial palsy, and Guillain-Barré syndrome among others (Connelly and DeWitt 1994). Haematological complications are more common, in particular haemolytic anaemia (3%) and thrombocytopenia (25-50%) (Jenson 2000), but also rarely aplastic anaemia, pancytopenia, and agranulocytosis. Other rare acute complications include myocarditis, pericarditis (Sabbatani et al. 2012), pancreatitis, interstitial pneumonia, rhabdomyolysis, and psychological complications (“Alice in Wonderland” syndrome). The strength of association of infectious mononucleosis with many of these complications is based on scattered case reports, and the evidence of causation in many instances is unconvincing (Jenson 2000). A thorough list of complications can be found at www.cdc.gov/epstein-barr/hcp.html.
4.12 Ongoing research

- The use of splenic volume to assess splenomegaly in infectious mononucleosis (proposed)
- Anaerobic antibiotics in infectious mononucleosis (proposed)
- Vaccination against Epstein-Barr virus (proposed—unaware of any active research)
- The pathogenesis of multiple sclerosis (several studies listed on ClinicalTrials.gov)
- Molecular analysis of Epstein-Barr virus related tumours and the role of the virus in ontogenesis (multiple studies listed on ClinicalTrials.gov)
4.13 Additional educational resources

4.13.1 Resources for healthcare professionals

- Candy B, Hotopf M. Steroids for symptom control in infectious mononucleosis. *Cochrane Database Syst Rev* 2006;3:CD004402 (a systematic review on the use of steroids in IM)

- Centre of Disease Control and prevention. Guidelines on laboratory testing for Epstein-Barr virus and infectious mononucleosis ([www.cdc.gov/epstein-barr/laboratory-testing.html](http://www.cdc.gov/epstein-barr/laboratory-testing.html)) (The CDC’s guidelines on diagnosing IM)

- NHS Clinical Knowledge Summaries on glandular fever ([http://cks.nice.org.uk/glandular-fever](http://cks.nice.org.uk/glandular-fever)) (a National Health Service resource on IM)

- BMJ best practice guidelines ([http://bestpractice.bmj.com/best-practice/monograph/123.html](http://bestpractice.bmj.com/best-practice/monograph/123.html)) (a helpful online resourse from the British Medical journal)

4.13.2 Resources for patients


4.14 Tips for general practitioners

General practitioners may see as many as 10 new cases of infectious mononucleosis a year (Candy et al. 2002). Although most patients will have mild symptoms, referral should be made to a secondary or tertiary centre in the following instances:

- Airway compromise
- Suspected splenic rupture
- Failure of supportive treatments (which may be indicated by the inability to swallow fluids or even saliva, and may occur in approximately 10% of patients) (Hoagland 1960b)
- Immunosuppressed or post-transplant patients
- Patients with infectious mononucleosis but negative for Epstein-Barr virus antibodies
SUPPLEMENTARY PUBLICATIONS

5.1 Response to Hanna Re: The diagnosis of infectious mononucleosis by Lennon et al. in a previous issue.
   Clinical Otolaryngology: PMID 22515717

5.2 Challenging the use of the absolute lymphocyte count in the diagnosis of infectious mononucleosis by analysis of a large cohort of monospot test results.
   Clinical Otolaryngology: PMID 23418980

5.3 In reference to "use of the lymphocyte count as a diagnostic screen in adults with suspected Epstein-Barr virus infectious mononucleosis".
   Laryngoscope: PMID 24610140

5.4 No new evidence to support the routine use of steroids in the treatment of infectious mononucleosis.
   Evidence Based Medicine: PMID 27099076
INTRODUCTION

This section of the thesis comprises of a number of minor publications that have come about, and are directly related, to the core papers of this study. Three of the articles are “Letters to the Editor”, one directly responding to a letter written in relation to the publication of Chapter 1 of this thesis. Another was a summary of our recent research in this area that was submitted to another Journal, in response to an article published on a similar subject. The third publication was again a response to research published by other authors on a similar topic to our paper in Chapter 1, but who had used the absolute lymphocyte count rather than the L/WCC ratio. We responded to this by re-running our calculations on our 1000 patients with Monospot results, and re-presented our findings as a short research note. The final article in this section is an invited review of a meta-analysis carried out to review the use of steroids in Infectious Mononucleosis.
5.1 Response to Hanna Re: The diagnosis of infectious mononucleosis by Lennon et al. in a previous issue.

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2. Department of Otolaryngology, Head and Neck Surgery, Mater Misericordiae University Hospital, Dublin

5.1.1 Background to the publication

In response to our publication of “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” in Clinical Otolaryngology, B. Hanna wrote the letter below to the editor (Hanna 2012). The authors main concern was proliferation of notion that IM and EBV are synonymous, in particular that this may lead the missed opportunities in the early diagnosis of HIV. Hanna’s letter can be seen on the following page and our response on the subsequent one.
Re: The diagnosis of infectious mononucleosis

3 January 2012

Sir,

I would like to draw attention to the common misconception that the diagnosis of infectious mononucleosis is synonymous with Epstein-Barr virus (EBV) infection.

The evolution of this misconception was illustrated by various authors and was subsequently challenged in a subsequent response to that paper. As explained by Lennox et al., the symptom triad of fever, pharyngitis, and cervical lymphadenopathy is described as "Glandular Fever" in 1889. The term "infectious mononucleosis" was coined by Sprunt and Evans in 1920 after finding lymphocytosis with atypical lymphocytes in six patients. The widely accepted definition of infectious mononucleosis, and remains, that of fever, pharyngitis, cervical lymphadenopathy, and lymphocytosis. It was subsequently discovered that EBV infection could cause infectious mononucleosis. It is recognized that this virus is responsible for 90% of cases. Laboratory tests became available for the heterophile antibody response produced by EBV (Paul-Bunnel test, Monospot®; Meridian Bioscience, Inc., Cincinnati, Ohio, USA) and were followed later by EBV serology. Many clinicians began to associate EBV infection with infectious mononucleosis to the exclusion of other possible causes of the symptoms. This is evident in the way that many clinicians use laboratory tests for EBV to correctly exclude infectious mononucleosis.

Other causes of infectious mononucleosis include cytomegalovirus, human herpes virus 6, toxoplasmosis and HIV. A correct diagnosis in the case of EBV-negative infectious mononucleosis is therefore important to two groups of people: pregnant women owing to the teratogenicity of some of these organisms and those in whom infectious mononucleosis is the manifestation of primary HIV seroconversion. A study in Brighton investigating missed opportunities for diagnosing primary HIV infection identified a number of patients in whom confusion with infectious mononucleosis compromised the potential for early diagnosis of HIV. Rose et al., in an earlier study in Boston, USA, found 9% of serum samples from patients with a negative monospot to test positive to HIV-1 plasma RNA.

Some authors have suggested that infectious mononucleosis should be redefined as a disease caused by EBV and that heterophile antibody-negative illnesses should be labelled "mononucleosis-like illnesses". This has only caused confusion about what term infectious mononucleosis means, and this term should not be used synonymously with EBV infection.

Infectious mononucleosis is a diagnosis based on the findings of fever, pharyngitis, cervical lymphadenopathy, and lymphocytosis. All subsequent tests are investigations to determine the cause of the illness. Positive tests for EBV identify that organism as the cause of the illness, but a negative result does not refute the clinical diagnosis of infectious mononucleosis. Further investigation is important if either pregnancy or HIV infections are the possibilities.

Conflict of interest

None to declare.

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References

3. Soderlund D., Paul D., Murphy G. et al. (2008) Missed opportunities for diagnosing primary HIV infection. Sex Transm. Infect. 84, 14–16

Figure 16: "Re: The diagnosis of infectious mononucleosis" by B. Hanna
5.1.2 Letter to the Editor

“Sir,

The aim of our study (Lennon et al. 2010) was not to further the misconception that EBV infection is synonymous with Epstein– Barr virus, but rather that the use of Lymphocyte to white cell count alone would lead to infectious mononucleosis (IM) being diagnosed as bacterial tonsillitis (BT) in up to one in seven case. Our paper was undertaken in response to an earlier paper by Wolf et al (Wolf et al. 2007) who suggested that a lymphocyte to white cell count (L/WCC) ratio of 0.35 could be used as a screening tool for IM. Our paper with much larger numbers demonstrated that the most appropriate diagnostic algorithm should remain, as suggested by Hoagland, that patients presenting with fever, pharyngitis and lymphadenopathy, with lymphocytosis and confirmed by positive serological test, can be diagnosed with IM (Hoagland 1975).

I do think that the above observation is an important one; however, we need to distinguish between the relatively common occurrence of an adolescent presenting with a sore throat against the rare occasions where these are accompanied by either the patient being pregnant or being in high-risk groups, as per Sudarshi et al (Sudarshi et al. 2008). In these cases, it would obviously be appropriate to order further investigations. As Hoagland warned over 50 years ago, disregarding the heterophile agglutination test would perpetuate considerable confusion and error, and we believe this summation still stands (Hoagland 1960a).”
5.2 Challenging the use of the absolute lymphocyte count in the diagnosis of infectious mononucleosis by analysis of a large cohort of monospot test results.

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5.2.1 Background to the publication

The use of a quick screening tool in infectious mononucleosis continued to interest a number of authors. One group proposed that the absolute lymphocyte count rather than the L/WCC be used as such a screening tool, and may in-fact be more accurate (Biggs 2011). They found that all patients with an absolute lymphocyte over 4 10*9/L had IM, whilst no patient with a diagnosis of BT had an elevated lymphocyte count. However their cohort was small and therefore we further investigated the hypothesis using our larger database. Again the paper by Biggs is in the next page and our response is on the following one.
Use of the absolute lymphocyte count in the diagnosis of infectious mononucleosis

5 July 2011

Sir,
A number of studies have been carried out, with one published in Clinical Otolaryngology, relating to the examination of the lymphocyte-to-white cell count ratio as a quick reference tool to aid in the diagnosis of infectious mononucleosis, whilst awaiting more definitive monospot (heterophile antibody test) or Epstein–Barr virus (EBV) serological testing. There are no reported studies within the literature detailing the use of the absolute lymphocyte count in this respect.

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Correspondence

Histogram of lymphocyte results

Fig. 1. Lymphocyte results of all patients studied.

One of the most widely cited criteria in the diagnosis of infectious mononucleosis is Hoagland’s criteria; at least 50% lymphocytes where at least 10% are atypical, in the presence of fever, pharyngitis and adenopathy, and confirmed by a positive serologic test. However, only 50% of patients with symptoms suggestive of infectious mononucleosis and a positive heterophile antibody test meet all of Hoagland’s criteria. More recently studies have suggested using the lymphocyte-to-white count ratio. One study suggested a lymphocyte-to-white count ratio of 0.35 or greater had a specificity of 72% and sensitivity of 84% for the detection of infectious mononucleosis. However, the use of the absolute lymphocyte count could be more accurate.

The blood results and notes of 81 patients admitted to the ENT department from 1 December 2010 to 4 April 2011 at Southampton General Hospital (SGH) were examined. They presented with a history of sore throat, fever and malaise; 33.3% (n = 27) had a diagnosis of quinsey, 12.3% (n = 10) infectious mononucleosis confirmed through monospot testing or EBV serology and the remaining 54.4% (n = 44) diagnosed as bacterial tonsillitis. When examining the absolute lymphocyte count and using the normal reference range of the laboratory at SGH (upper limit of normal being 4 x 10^9/L), it was found that all patients diagnosed with infectious mononucleosis had a high lymphocyte count (level above the normal reference range) as seen in Fig. 1. On examination of those patients without infectious mononucleosis, none had a lymphocyte count above the normal reference range.

This study has highlighted a correlation between a raised absolute lymphocyte count (>4 x 10^9/L) and the presence of a positive monospot or EBV serology test, indicating the diagnosis of infectious mononucleosis. A recommendation would be to use the absolute lymphocyte count as a quick reference tool in the diagnosis of infectious mononucleosis whilst awaiting definitive monospot or EBV serological testing, which depending on the laboratory can take up to 48 h to receive.

Conflicts of interest

None declared.

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References


Figure 17: Absolute lymphocyte count and the diagnosis of IM, T.C. Biggs
5.2.2 Letter to the Editor

“Sir,

We read with interest the correspondence from T. C Biggs (Biggs 2011) in a previous issue espousing the use of a raised absolute lymphocyte count (> 4 x 10⁹/L) as a quick reference tool in the diagnosis of infectious mononucleosis. This is a further step in the ongoing debate on a rapidly available screening tool, whilst waiting for confirmation of infectious mononucleosis (IM) by either definitive monospot or EBV serological testing. This debate was initiated by a study by Wolf et al (Wolf et al. 2007) recommending that the lymphocyte to white cell count (L/WCC) ratio of 0.35 should be used as an indicator to decide whether mononucleosis spot tests should be requested.

With much larger numbers, our group demonstrated that L/WCC ratio is not sufficient to diagnose or exclude infectious mononucleosis (Lennon et al. 2010) and that one in seven of the patients with infectious mononucleosis would be missed leading to mismanagement and potentially increased morbidity.

Biggs found that all the patients with glandular fever had a high white cell count greater than the upper limit of normal of 4 x 10⁹/L. However, there were only 10 patients in this group. Using the data already collected from our 1000 patients in the previous study, we calculated the sensitivity, specificity, positive predictive value and negative predictive value as 61.8%, 96.8%, 95% and 71.7%, respectively (Table 12)(Fig 18).

Table 12: Sensitivity, Specificity, PPV and NPV for absolute lymphocyte count in IM

<table>
<thead>
<tr>
<th></th>
<th>TP/TP &amp; FN</th>
<th>TN/TN &amp; FP</th>
<th>TP/TP&amp;FP</th>
<th>TN/TN&amp;FN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>309/500</td>
<td>484/500</td>
<td>309/325</td>
<td>484/675</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PPV</td>
<td></td>
<td></td>
<td>95%</td>
<td></td>
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<tr>
<td>NPV</td>
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<td>71.7%</td>
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</table>
Using a cut-off of $4 \times 10^9/L$, there were relatively very few patients (16) with false-positive results, that is, those with a negative monospot but a high absolute lymphocyte count, giving a high specificity (96.8%) and positive predictive value (95%). There was however, still a large amount of patients (191) with absolute lymphocyte counts below $4 \times 10^9/L$ that had positive monospot results. We therefore would continue to advocate that the L/WCC ratio or high absolute lymphocyte count should be used in conjunction with the Hoagland criteria (Hoagland 1975).”
5.3 In reference to "use of the lymphocyte count as a diagnostic screen in adults with suspected Epstein-Barr virus infectious mononucleosis".

Paul Lennon¹,², James Paul O’Neill², John E. Fenton¹, Tadhg O’Dwyer²

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5.3.1 Background to the publication

This article once again came about due to the ongoing enthusiasm for the use of an absolute white cell count over 4x10⁹/L as a diagnostic screening tool. Biggs et al carried out a larger retrospective review of 726 patients that had Monospot testing (Biggs et al. 2013). They found that a lymphocyte count of ≤4 x 10⁹/L was associated with negative Monospot result in 99% of patients. They concluded that this parameter was a highly reliable negative predictor of EBV-related IM and suggested limiting routine Monospot and/or serological testing to suspected cases of EBV-related IM in patients with a lymphocyte count >4 x 10⁹/L. These results again differed substantially from the findings that we had published, and therefore we wrote a letter to the editor warn of the potential pitfalls of such an approach.
5.3.2 Letter to the Editor

“Dear Editor,

We read the recent article by Biggs et al (Biggs et al. 2013) on the use of the absolute lymphocyte count in the diagnosis of infectious mononucleosis. We carried out a similar study on the use of the lymphocyte to white cell ratio (Lennon et al. 2010), and later reanalyzed our data using an absolute lymphocyte count above 4 (Lennon et al. 2013a), in response to an earlier study carried out by Biggs et al (Biggs 2011). We would like to highlight the divergence in results between our study and the current study by Biggs et al. The authors analyzed 50 patients with positive monospot tests, and found a negative predictive value of 99%, specificity of 94%, and sensitivity of 84%. Our cohort had 500 positive monospot results, and we found a negative predictive value of 71.7%, a specificity of 96.8%, and sensitivity of 61.8%. Our article demonstrated that a much larger number of patients had positive monospots with a lymphocyte count of <4 (191 cases), suggesting that the use of the lymphocyte count is associated with a high rate of false-negative results.

As with the paper by Wolf et al (Wolf et al. 2007) previously, we recommend the continued use of the Hoagland criteria (Hoagland 1975) for diagnosis of infectious mononucleosis, as the modest saving incurred with the abandonment of the monospot test may lead to the provision of misinformation on recovery, including the avoidance of contact sports, and potentially far greater costs to both the patient and the medical institution involved.”
5.4 No new evidence to support the routine use of steroids in the treatment of infectious mononucleosis.

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5.4.1 Background to the publication

Evidence-Based Medicine is a review journal of the British Medical Journal. Its stated goal is to systematically search “a wide range of international medical journals applying strict criteria for the validity of research. Content is critically appraised then the most clinically relevant articles are summarised into an expert commentary focusing on the papers clinical applicability (Evidence-Based Medicine 2016)”. The Cochrane review suggests that published systematic reviews be regularly updated (Cochrane Review updates 2016). As steroid use in IM was a component of the review we had carried out published by the BMJ, we were invited to comment on the most recent update of the systematic review of steroid use in IM by Candy et al (Candy and Hotopf 2006).
5.4.2 Invited Review

5.4.2.1 Context

Infectious mononucleosis is a non-genetic syndrome, defined by the classic triad of fever, pharyngitis and cervical lymphadenopathy, where lymphocytosis is also present. Over 90% of cases are caused by the Epstein Barr virus, which results in a subclinical infection in childhood and to which 95% of the population have seroconverted by adolescent (Lennon et al. 2015). Infection after this time will result in IM, which has been shown to be a more severe illness than simple bacterial tonsillitis (Lennon et al. 2013b). Therefore, attempts are ongoing to expand the treatment armamentarium (Lennon et al. 2014a). Some early reports supported the use of corticosteroids in the treatment of IM, but other trials showed these effects to be short lived. A Cochrane review was undertaken in 2006, which concluded that there was insufficient evidence and the trials were too few, heterogeneous, and of poor quality to recommend steroid treatment for symptom control in glandular fever (Candy and Hotopf 2006). This systematic review, carried out by different authors, is an update on the initial study and subsequent updates in 2009 and 2011.
5.4.2.2 Methods

This was a review of randomised controlled trials examining the effects of steroids in IM. The patients could be of any age with confirmed IM by serology or monospot test. The intervention was steroids of any dose, duration or route of administration. The comparators were either placebo, standard of care or another agent. The outcomes included improvement in health and duration of symptoms, but also adverse events due to steroid use. The authors used the standard methodological procedures set out by the Cochrane review. As this is an update, the authors searched for new studies carried out between 2011 and 2015. The results depended on the individual study with odds ratio (OR), 95% confidence interval (CI) or mean difference (MD) reported.
5.4.2.3 Findings.

The authors reported no new studies that met the inclusion criteria for this update. One hundred and eighty-six search results were found to be irrelevant. There were no studies found, ongoing or completed, in several trial registries. In fact, no new studies have been added in any of the three updates that have been undertaken thus far. The original review included 7 RCTs with a total of 362 patients. The quality of the evidence was generally found to be poor and the heterogeneity between trials prevented a combined analysis. For instance, from the intervention perspective there was a wide range of dose regimes, ranging from a single dose to a 12-day course. On the comparator side, 4 studies had a placebo arm, but others compared steroids to aspirin or combined steroids with an antiviral. The authors found evidence from two trials that steroids were effective in reducing the symptoms of sore throat initially but not after 12 hours of treatment. Evidence on other symptoms was limited and a number of adverse events, including peritonsillar abscess, empyema and the development of diabetes mellitus were reported in patients treated with steroids. The authors concluded that there is not enough evidence to the efficacy of corticosteroid treatment for symptom control in people with otherwise uncomplicated glandular fever.
5.4.2.4 Commentary

The Cochrane website suggests two yearly updates of its studies in order to maintain a contemporary status (Cochrane Review updates 2016). This is the third update of the original systematic review and meta-analysis carried out by Candy et al in 2006. There were no new studies found in the interim and the level of evidence remains poor. The number of studies, and number of patients in each, resulted in many of the analyses having wide confidence intervals with little assurance to the real effect of steroids in IM. Anecdotally there has been an increase in the use of steroids to treat IM in the emergency room setting (Lennon et al. 2014a). This may have resulted from a more recent Cochrane review on a similar topic, which concluded that corticosteroids increased the likelihood of both resolution and improvement of pain in participants with sore throat (Hayward et al. 2012). Although this review excluded studies with IM, many patients are likely to receive steroids prior to a diagnosis of IM being made in an emergency room setting. This review again reiterated that there is no good data for the routine use of steroids and highlights a number of possible adverse events that may be associated with their use.
5.4.2.5 Implications for practice

Consideration for the use of steroids in IM should be reserved for patients with impending airway compromise. However their routine use, without a clear evidence base, remains widespread (Thompson et al. 2005). Clinical equipoise remains important and additional studies, with greater numbers of patients and improved study designs, are required prior to a comprehensive recommendation. There is currently insufficient evidence to recommend corticosteroid treatment for symptom control for people with otherwise uncomplicated IM.
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