Infectious mononucleosis in university students: evaluation of the clinical features and consequences of the disease.

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40 word Summary
IM resulted in marked reductions in student study time, social activities and physical exercise, and sustained hypersomnia. Females were more likely to discontinue their studies following IM, and reported more prolonged fatigue. Increased γδ T-cells persisted into the convalescent period.

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SUMMARY

Background Infectious mononucleosis (IM) is common among university students and the present study was undertaken to analyze the clinical features and sequelae of the disease in a cohort of students at Edinburgh University.

Methods Consecutive IM cases were recruited between 2000 and 2002 through the University Health Service (UHS) after diagnosis of IM.

Results IM resulted in marked reductions in student study time, physical exercise, non-exercise related social activities; and sustained increases in reported hours of sleep. Disease profile differed between the sexes, with significantly more females reporting fatigue, which was more likely to be prolonged (p=0.003) and to lead to loss of study time (p=0.013). Female cases were more likely to discontinue their studies following IM (16% vs. 0% p=0.056). Within the typically raised lymphocyte counts in IM we identified a raised \( \gamma \delta \) T-cell component that may contribute to the disease pathogenesis.

Conclusions IM results in substantial morbidity among university students, reported as more profound in females, and impacting on academic, physical exercise and social activities. Immunization to prevent IM and strategies to reduce post-IM disability would be beneficial in this population.
INTRODUCTION

Infectious mononucleosis (IM) is an acute self-limiting disease caused by primary Epstein-Barr virus (EBV) infection. EBV persistently infects over 90% of adults worldwide and is usually transmitted subclinically between young children via saliva; however, if primary infection is delayed until adolescence or early adulthood, IM develops in 25-74% of cases (1;2). IM classically presents with sore throat, fever, lymphadenopathy, and fatigue, with earlier studies, based on observation of hospitalised males, indicating complete recovery in 2-6 weeks (3;4). However, later surveys, based in general practice, have emphasised the frequent occurrence of prolonged fatigue (5;6).

During IM, EBV infects B-lymphocytes in the oropharynx, inducing their proliferation and dissemination. This invokes massive proliferation of virus-specific, CD8+ cytotoxic T-lymphocytes which colonise lymphoid tissues and invade other organs. It is thought that cytokines produced by these T-cells, particularly interferon γ and tumour necrosis factor α, are responsible for the clinical picture of IM (7-9) and a correlation between the level of T-cell activation and severity of symptoms has been noted (10). However, the pathogenesis of IM remains unclear. Sexual activity is a strong risk factor for both EBV seroconversion and IM in young adults (11), possibly by promoting transmission of large amounts of virus, triggering the exaggerated T-cell response. Genetic factors also play a part in IM development since certain cytokine gene polymorphisms increase susceptibility to EBV infection (12), and we have recently identified two polymorphisms in the HLA class 1 gene that predispose to IM (13).

The incidence of IM not only varies with age but also with social status and country of residence. It is common in Western societies, particularly among high socioeconomic groups, where children are relatively protected from early EBV infection (14;15). In contrast, in
developing countries, where almost all children silently seroconvert to EBV at an early age, IM is rare (16).

Complications are unusual in teenagers and young adults, although prolonged fatigue is a common sequel (6). Delayed diagnosis, atypical presentation and severe disease are more frequent in older patients in industrialised societies and recently an increase in hospital admissions has been reported (17;18).

Several studies (6;19-23) have examined the clinical features of IM or its long term sequelae, particularly its association with chronic fatigue syndrome (CFS), but none has studied IM in university students and addressed the consequences of the disease to their studies. The present study of Edinburgh University students describes the clinical features and consequences of IM among this young adult population. We demonstrate a number of important abnormalities, some of which persisted for several months.

METHODS

Recruitment of Cases and Controls

Participants were recruited during a study on EBV undertaken at Edinburgh University from 2000 to 2002, as previously described (1;11). Briefly, students registering at the UHS at the start of 4 year degree courses were invited to participate. EBV seronegative students were asked to return to the UHS if symptoms of IM developed, so that EBV serological tests could be performed. Students presenting to the UHS with clinical symptoms of IM, who were not in the original study group, could be included if they fulfilled the serological inclusion criteria. Diagnosis required either a positive anti-VCA IgM with demonstrable seroconversion to IgG anti-VCA, or, a positive monospot test with demonstrable seroconversion to IgG anti-VCA, or a positive monospot and anti-VCA IgM. Students with negative monospot and negative anti-VCA IgM tests suffered from ‘IM-like’ illnesses, and formed a comparison group for flow cytometric analysis (see below).
Fifty-seven IM cases completed a lifestyle questionnaire at diagnosis and were subsequently followed up for 5-6 months. The controls (n=58) were selected from EBV seronegative students, such that overall age, sex, and year of study had a similar distribution to the case group. Controls were included in the analyses of laboratory and lifestyle data to track the return of case values to normal levels. The study was approved by the Lothian Ethics Committee, and each case and control gave signed, informed consent.

**EBV Serology**

Anti-VCA IgG and IgM were determined by routine immunofluorescence, and heterophile antibodies were detected by the monospot test (Microgen im absorption kit, Camberley, Surrey).

**Full Blood Count**

Haemoglobin, total lymphocyte, monocyte, neutrophil and platelet counts were performed using an automated counter (SE-9000, Sysmex, Milton Keynes) in the Department of Haematology, Edinburgh Royal Infirmary (ERI), and compared with normal ranges set by that Department.

**Liver Function Tests (LFT)**

Alanine transaminase (ALT), alkaline phosphatase (ALKP), gamma glutamyl transferase (GGT), bilirubin and albumin were measured using an automated analyser (Olympus AU640, Watford) by the Department of Clinical Biochemistry, ERI, and compared with normal ranges set by that Department.

**Flow Cytometry**

Lymphocyte subset analysis was performed on 25 IM patients, 12 healthy controls, and 9 ‘IM-like’ cases. Peripheral blood mononuclear cells (PBMC) were separated by density centrifugation and stained for the following antigen combinations: CD8+/CD3+, CD4+/CD3+, CD56+/CD3−, CD19+/CD3− and CD3−/γδ+ using directly labelled antibodies (Becton
Dickinson, [BD]) and analysed on a BD Facs Caliber. Data analysis was performed using Cell Quest acquisition and analysis software (BD). The proportion of cells positive for both markers was determined, and lymphocyte numbers for each subset were calculated from the peripheral blood total lymphocyte count.

**Viral Load**

Viral load was measured using a semi-quantitative technique following the methodology of Stevens (24), exactly as previously described (10).

**Assessment of Fatigue and Stress**

IM cases and controls completed a self-rated fatigue questionnaire, which quantified their ability to perform specific tasks (e.g. getting up, dressing, washing) at the worst stage of the illness, and the extent to which the illness had affected their ability to exercise, study and participate in social activities. A numerical rating scale from zero “no fatigue” to ten “as bad as you can imagine” based on the brief fatigue inventory scored the student’s perception of their fatigue severity at each visit (25). Stressful events (death/serious illness in close relative or partner; broken marriage/steady relationship; financial crisis; part-time job taken to relieve financial pressures; problems with exams or studies) and psychiatric illnesses in the 6 months prior to IM development were also recorded.

**Statistical Analysis**

Non-parametric tests were used to avoid distributional assumptions. The Spearman test of correlation was used to investigate association between two continuous variables, whilst the Mann-Whitney-U test was used to investigate the difference in continuous variables between groups. The chi square and Fisher’s exact tests were used to examine differences in categorical data between groups.
RESULTS

IM cases and controls

The 57 IM cases and 58 controls recruited were similar with respect to sex: 46% (n=26) male among IM cases, 40% (n=23) among controls, and age (median age: IM cases 20.0 years; controls 20.1 years).

Complete EBV serology results from the first UHS visit were available for 52 of the 57 cases. At diagnosis, 92% of IM cases (48/52) had anti-VCA IgM. Amongst 46 IM cases retested within a month of diagnosis, 45 (98%) were positive. IgM antibodies declined over time with 69% (18/26) positive at 1-2 months, 37% (7/19) at 2-4 months, and 6% (1/18) by 5-7 months after diagnosis.

A similar pattern was seen with the monospot test with 51/52 (98%) positive at diagnosis, and the sole negative having become positive when tested 9 days later. Heterophile antibodies declined with time, with 69% (18/26) positive at 1-2 months, 47% (9/19) at 2-4 months and 28% (5/18) at 5-7 months after diagnosis.

IM symptoms

The three most common presenting symptoms were sore throat (77%), fatigue (65%) and awareness of cervical lymphadenopathy (54%) (Figure 1). Sore throat lasted for a median of 7 days and was experienced by 55/57 (96%) cases at some stage of the illness. In 32 of 57 (56%) this was severe enough to cause an altered diet, with four (7%) experiencing problems swallowing liquids. Cervical lymphadenopathy was evident in 88% of IM cases. Overall 16% had splenomegaly and 21% hepatomegaly, but among those examined within 2 weeks of disease onset 35% had splenomegaly and/or hepatosplenomegaly.

All students experienced fatigue at the worst stage of their illness; however, there was marked variation in severity. Overall 79% (45/57) of students reported reduced walking distance with 37% (21/57) being unable to leave their home. Females were significantly more likely to
report fatigue at 6 months after diagnosis than males (34% vs. 5% respectively, p=0.012, Table 1). Students reporting stressful events (n=24) or mental illness (n=12) in the 6 months prior to IM were more likely, although not significantly so, to experience a longer duration of fatigue (medians 101 and 114 days) than students not so affected (medians 63 and 66 days). In females this trend was more pronounced, with a median fatigue duration of 194 days in those reporting prior stressful events vs. 80 days for those without (p=0.03), and of 263 days in those with prior mental illness vs. 109 days for unaffected females (p=0.17).

IM cases took significantly less exercise than controls until 30-60 days after diagnosis (p=0.04) (Table 2). However, cases reported higher pre-morbid exercise levels than controls (median 4 hours per week), such that cases had not returned to their reported pre-morbid exercise levels at the final follow-up 150 days post-diagnosis. At 5 months after diagnosis, females were more likely to report a reduced ability to exercise than males (33% vs. 8%) although the difference was not significant (p=0.11). Males showed a significant positive correlation between weekly hours of exercise before the onset of illness and the subsequent duration of fatigue (p=0.03). Non-exercise related social activities were severely curtailed by IM and were reduced compared with controls at all follow-up visits. At 5 or more months after diagnosis cases spent 5 hours per week less than controls on social activities, which is in keeping with their reported on-going requirement for additional sleep (Table 2).

Forty-two (75%) of 56 IM cases missed timetabled classes (median 11 hours, range 0-300 hours); the proportion being significantly greater in females (87%) than males (60%, p=0.02). Five female IM cases abandoned their course following IM; two left university completely and three planned to repeat the academic year. There was a significant association between duration of fatigue and number of hours missed from classes (p=0.003), present among females (p=0.02) but not males (p=0.17). The severity of initial disability predicted the
number of classes missed (Table 3), and being unable to walk 100m at the time of most severe symptoms predicted longer duration of fatigue (94 days vs. 55 days, p=0.04).

The initial numerical fatigue score correlated positively with subsequent duration of fatigue (p=0.023) and the number of missed classes (p=0.001) (Figures 2 and 3). The latter result being significant for each sex (females p=0.015, males p=0.026).

**Laboratory Findings**

Seventy-four percent of cases, but none of the controls, had lymphocyte counts above the normal range (1.5 – 4.0 x10^9/l) at diagnosis. The median lymphocyte (5.8 x10^9/l) and monocyte (1.35 x10^9/l) counts of IM cases at diagnosis differed significantly from median control counts (1.55 and 0.42 x10^9/l respectively, p<0.001 for both) and this difference persisted for up to 15 days for monocytes and 30 days for lymphocytes. In contrast, median neutrophil (2.77 x10^9/l) and platelet counts (188 x10^9/l) were significantly lower in IM cases at diagnosis compared with controls (3.28 x10^9/l for neutrophils (p=0.015); 252 x10^9/l for platelets (p=0.001)). At diagnosis 10/47 (21%) had platelet counts below the normal range (150 x10^9/l) and four students (9%) had neutrophil counts of less than 1 x10^9/l (0.5 – 0.9 x10^9/l). All but one IM case had a platelet count within the normal range by one month after diagnosis, whereas, significantly low neutrophil counts persisted for 2 months. Haemoglobin values differed significantly between male (median 154 g/l) and female (median 132.5 g/l) controls (p<0.0001). Male IM cases had lower Hb values at diagnosis (median 143.5 g/l), than controls (p=0.006), and remained significantly lower than control males for up to 30 days after diagnosis (141.0 g/l, p=0.0005), whereas, the values in female IM cases at diagnosis (131.5 g/l) did not differ significantly from female controls (p=0.68). There was evidence that students reporting severe sore throat, defined as unable to swallow a soft diet, had higher total white cell counts (p=0.04) and monocyte counts (p=0.01) than those without severe sore throat, and that among males, but not females, higher monocyte (p=0.033; p=0.25) and total
lymphocyte (p=0.064; p=0.97) counts at diagnosis were positively correlated with missed university classes.

LFT were performed for all cases and 53/58 controls. Overall 65% of cases and 21% controls had one or more elevated values (p<0.001). In 27 IM cases LFT were performed within 2 weeks of diagnosis and 21/27 (78%) of these had one or more abnormal results. Twenty (74%) of the 27 IM cases had elevated ALT, fourteen (52%) elevated GGT, 5 (19%) elevated ALKP, and 3 males (11%) had elevated bilirubin. Normal values for GGT differed significantly between the sexes and so were analysed separately in males and females. Eleven (41%) of 27 IM cases had ALTs greater than twice the upper limit of normal and 5 (19%) had results greater than 5 times this value. Eleven of 53 controls (21%) had either an elevated bilirubin (9 males) or GGT (1 male, 1 female). None of the controls had abnormal ALKP or ALT, and only one had more than a trivial abnormality, with GGT 2.7 times the upper normal limit. There was no significant difference in the proportion of cases and controls with a raised bilirubin (p=0.62).

Flow cytometric analysis of blood leucocytes in IM

Acute IM cases had significantly higher median numbers of total lymphocytes than healthy controls (p<0.001) and the ‘IM-like’ group (p<0.001). The acute IM cases had significantly higher numbers of CD3+ (p<0.001), CD8+ (p<0.001), CD56+ (p<0.001) and γδ T-cells (p<0.001), but not CD19+ (p=0.72) or CD4+ (p=0.86), subsets, than controls (Figures 4a-g).

Convalescent cases had higher CD56+ and γδ T-cell counts than controls or “IM-like cases” and when convalescent samples were analysed according to time since diagnosis, total lymphocyte counts and CD56+ NK cells remained significantly higher than controls for up to 4 months, CD3+ and CD8+ T-cells for <15 days, and γδ T-cells for >150 days.
DISCUSSION

In this study we recorded the type and duration of symptoms in a cohort of 57 university students with IM. Cases were within the classic age range for IM (18-27 years), and the great majority had sore throat, fatigue or complained of lymphadenopathy. However these symptoms are not distinctive for IM, since during the study only 28% of students with suggestive symptoms that underwent serological testing at the UHS were confirmed as IM. At the initial GP visit the monospot test may be more sensitive (98% of 52 cases positive) than the IgM test (92% positive) for IM diagnosis, although this did not attain significance (p=0.51). Thus, although the monospot test is not specific for EBV antigens, reliance on the IgM test alone would have resulted in 8% of IM cases being missed at the initial GP visit.

Complications apart from fatigue were unusual; two students were admitted to hospital, one with jaundice, and another with severe dysphagia. Two further students required medical care, without admission, for dysphagia. Acute symptoms lasted a median of seven days in both sexes; however, self-reported fatigue lasted longer in females (median 118 days) than males (median 49 days). In this study we restricted our analysis to the acute disease and did not attempt to define risk factors for CFS as carried out by White et al (23). However we have highlighted several interesting clinical features, particularly differences in disease profile between the sexes. Comparison of symptoms between males and females revealed that self-reported IM-associated fatigue was significantly more common, severe and long-lasting in females. This resulted in females taking less exercise during their illness, missing more study, and being more likely to discontinue their studies. Thus the consequences of IM were more serious in females who would perhaps have benefited from targeted interventions to reduce morbidity (26).

Severe fatigue has long been associated with IM and, along with the other symptoms of IM, is generally assumed to be caused by cytokines released by the large numbers of circulating,
activated, CD8$^+$ T-cells typically found in acute IM (7-9;23). However, although we did not measure serum cytokine levels directly, we found no association between total numbers of peripheral blood lymphocytes, CD4$^+$, CD8$^+$ or NK lymphocyte subsets and fatigue severity or duration. EB viral load (VL) in PBMC and did not correlate with disease severity, fatigue duration or missed classes. Balfour et al (19) found a correlation with a composite score of physical activity limitation and symptom intensity with whole blood VL, but did not state whether there was a correlation between disease severity and VL in PBMC.

At diagnosis we recorded the classical high lymphocyte counts, mainly accounted for by CD8$^+$ T-cells, but also containing raised numbers of CD56$^+$NK cells and γδ T-cells. Twenty-one cases had asymptomatic thrombocytopenia, a common but not universal finding (27;28), and none developed the rare hemorrhagic manifestations (29).

High CD8$^+$ T-cell counts returned to normal levels by 30 days after diagnosis, but other abnormalities persisted longer, with low neutrophils up to 60 days, and high NK cells up to 4 months, and high γδ T-cells for over 5 months. These raised values did not correlate with VL, illness duration, fatigue or hours missed from university. High γδ T-cells in IM has been reported previously in a study on 10 cases (30), and is interesting because these cells, although very similar to αβ T-cells, can recognise non-peptide antigens and possess innate effector functions (31). They are potent antigen presenting cells in vitro, with the ability to present peptide antigens to αβ T-cells (32) and thus may play a key role in IM pathogenesis by activating CD8$^+$ T-cells and inducing the characteristic massive response associated with IM.

This study identifies substantial IM-related morbidity, particularly in female patients. Thus primary preventative strategies, particularly immunization, and tertiary preventative strategies to prevent disability, such as graded activity and lifestyle management, may reduce morbidity in this population (26;33).
Acknowledgements

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Potential conflicts of interest: all authors; no conflicts.
References


### Table 1 Disease profile in male and female IM cases

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<th>Symptom</th>
<th>Male</th>
<th>Female</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue &gt; 180 days</td>
<td>1/21</td>
<td>10/29</td>
<td>0.012</td>
</tr>
<tr>
<td>Median duration fatigue</td>
<td>49 days</td>
<td>118 days</td>
<td>0.003</td>
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<tr>
<td>Median fatigue score at worst</td>
<td>7</td>
<td>8</td>
<td>0.016</td>
</tr>
<tr>
<td>Missed classes (median hours)</td>
<td>3</td>
<td>16</td>
<td>0.013</td>
</tr>
<tr>
<td>Discontinued studies</td>
<td>0/26</td>
<td>5/31</td>
<td>0.056</td>
</tr>
<tr>
<td>House bound</td>
<td>7/26</td>
<td>14/30</td>
<td>0.128</td>
</tr>
<tr>
<td>Reduced walking distance</td>
<td>18/26</td>
<td>27/31</td>
<td>0.099</td>
</tr>
<tr>
<td>Unable to eat soft diet</td>
<td>8/26</td>
<td>14/31</td>
<td>0.266</td>
</tr>
<tr>
<td>Viral Load at diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>genomes per 10^6 peripheral blood mononuclear cells</td>
<td>3498</td>
<td>8766</td>
<td>0.362</td>
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Table 2 Median hours per week\(^\dagger\) spent on different activities in controls and in IM cases by time elapsed since diagnosis

<table>
<thead>
<tr>
<th>Activity</th>
<th>Diagnosis</th>
<th>&lt;15</th>
<th>15-30</th>
<th>30-60</th>
<th>60-120</th>
<th>&gt;150</th>
<th>Control</th>
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<tr>
<td></td>
<td>n=57</td>
<td>n=37</td>
<td>n=27</td>
<td>n=20</td>
<td>n=32</td>
<td>n=58</td>
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<tr>
<td>Academic work *</td>
<td>0 n=43</td>
<td>15 n=27</td>
<td>22.3 n=34</td>
<td>24.5 n=24</td>
<td>24.5 n=16</td>
<td>27 n=25</td>
<td>26 n=56</td>
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<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p=0.119</td>
<td>p=0.321</td>
<td>p=0.346</td>
<td>p=0.675</td>
<td></td>
</tr>
<tr>
<td>Exercising</td>
<td>0 n=57</td>
<td>0 n=31</td>
<td>0 n=31</td>
<td>0 n=31</td>
<td>0 n=31</td>
<td>2 n=31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p=0.872</td>
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</tr>
<tr>
<td>Social activities</td>
<td>0 n=57</td>
<td>4 n=36</td>
<td>5 n=36</td>
<td>6 n=36</td>
<td>10 n=36</td>
<td>7 n=30</td>
<td>12 n=57</td>
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<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p=0.003</td>
<td>p=0.220</td>
<td>p=0.002</td>
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<tr>
<td>Sleeping (^\dagger)</td>
<td>- n=57</td>
<td>10 n=36</td>
<td>9 n=36</td>
<td>8 n=36</td>
<td>8.5 n=19</td>
<td>8 n=31</td>
<td>7 n=57</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
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</table>

* Timetabled classes and private study
\(^\dagger\) For hours sleeping, per 24 hours
Table 3 Severity of initial disability predicted number of hours of University classes missed due to illness

<table>
<thead>
<tr>
<th>Feature</th>
<th>Hours missed if feature present</th>
<th>Hours missed if feature absent</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Not able to leave home during worst symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>35</td>
<td>3.5</td>
<td>p=0.003</td>
</tr>
<tr>
<td>Male 7/25</td>
<td>17</td>
<td>1</td>
<td>p=0.111</td>
</tr>
<tr>
<td>Female 14/30</td>
<td>56</td>
<td>10</td>
<td>p=0.041</td>
</tr>
<tr>
<td><strong>Not able to wash and dress during worst symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>56</td>
<td>8</td>
<td>p=0.010</td>
</tr>
<tr>
<td>Male 3/25</td>
<td>39</td>
<td>2</td>
<td>p=0.052</td>
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<tr>
<td>Female 5/29</td>
<td>115</td>
<td>13.5</td>
<td>p=0.069</td>
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<tr>
<td><strong>Reported reduced walking distance during worst symptoms</strong></td>
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<tr>
<td>All</td>
<td>16</td>
<td>0</td>
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<tr>
<td>Male 18/25</td>
<td>7</td>
<td>0</td>
<td>p=0.097</td>
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<tr>
<td>Female 27/31</td>
<td>35</td>
<td>4.5</td>
<td>p=0.039</td>
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Legends for Figures

Figure 1 Presenting symptoms in IM

Figure 2 Initial fatigue score correlates with the duration of fatigue p=0.023

Figure 3 Initial fatigue score correlates with the number of hours of timetabled classes missed p<0.0001

Figures 4a-g Lymphocyte subset analysis in acute IM, convalescence, controls and “IM-like” illness

a Total lymphocytes, b T-lymphocytes, c CD8\(^+\) lymphocytes, d CD4\(^+\) lymphocytes, e CD56\(^+\) NK cells, f B-lymphocytes, g \(\gamma\delta\) T-lymphocytes
initial fatigue score

missed university classes (hours)