BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2021, VOL. 78, NO. 3, 107–116
https://doi.org/10.1080/09674845.2021.1903683

REVIEW ARTICLE

Infectious Mononucleosis: diagnosis and clinical interpretation

P Naughton[a,b], M Healy[a], F Enright[c] and B Lucey[a]

[a]Department of Biological Sciences, Munster Technological University, Bishopstown, Cork, Ireland; [b]Department of Haematology, Mercy University Hospital, Cork, Ireland; [c]Department of Paediatrics, Mercy University Hospital, Cork, Ireland

ABSTRACT

EBV is the sole causative agent of the acute illness in humans described either as infectious mononucleosis (IM), or glandular fever. IM, when not clinically silent, can present in patients with at least two of the classic triad of symptoms of fever, pharyngitis, and lymphadenopathy. Challenges for the clinician arise when atypical cases present. Early, accurate and informed laboratory test results are vital for diagnosis, appropriate treatment, and management. A key challenge for the practitioner, particularly in cases where the illness can present atypically, is distinguishing bacterial tonsillitis infections from early acute IM. The ability to draw on timely, clear, and insightful laboratory results to distinguish viral from bacterial infection is vital. Correct and prompt diagnosis of IM can help prevent the unnecessary administration of antibiotics and mitigate the need for other expensive exploratory tests in cases of IM that present with splenomegaly, lymphadenopathy, or suspect haematological conditions. Good communication between the requesting clinician and those carrying out the investigative process, and between the different laboratory departments involved, is good practice and would ultimately benefit the patient. This communication will comprehensively review the aetiology, clinical presentation, and laboratory findings in IM with a view to promoting further research and so derive a standard diagnostic algorithm of the condition.

ARTICLE HISTORY
Received 25 January 2021
Accepted 11 March 2021

KEYWORDS
Infectious mononucleosis/ glandular fever; Epstein-Barr virus; diagnostic algorithms; laboratory diagnosis

Introduction

The infectious disease caused by the Epstein Barr virus (EBV) was first coined 'Infectious Mononucleosis' (IM) by Sprunt and Evans in 1920. Their findings of a consistent mononuclear leucocytosis in reaction to acute infection in several patients exhibiting similar signs and symptoms led them to the correct belief that this constituted a distinct clinical syndrome [1]. It was only following the identification and discovery of the EBV some 44 years later and the subsequent linking of this virus to IM that a clear and proper understanding of this unique clinical syndrome could finally be elucidated. Even today there is limited consensus in the literature regarding an unambiguous definition of IM and the pathogens involved. Some literature states that though EBV infection is the main cause of IM, other human herpes viruses, principally human cytomegalovirus (CMV) [2–7] and to a lesser extent Roseola virus (HHV-6), are also implicated in lower percentages of cases [8,9].

The scope of the current review is to discuss true IM only, i.e. only those cases where EBV is identified to be the sole causative agent for the disease. Other infections with similar clinical symptoms to IM but linked to other pathogens should be considered as infectious mononucleosis-like infections to distinguish between the two [10,11].

EBV is named after Anthony Epstein and Yvonne Barr, who in 1964 identified the virus in lymphoma blast cells from tissue samples of patients with Burkitt's lymphoma. The latter was originally reported as an aggressive sarcoma often involving the jaws of children which was endemic in Uganda and neighbouring countries [12]. This originally termed 'sarcoma' was soon re-classified as a lymphoma [13–16] and later named Burkitt's lymphoma. Burkitt sent biopsy specimens from Uganda to Epstein and Barr who cultured malignant lymphoblasts from the biopsies and with the aid of electron microscopy described the first human oncogenic virus that now bears their name (Figure 1).

Clinical presentation and treatment of IM

The clinical picture in the condition now classified as IM can vary depending on how the disease presents. Symptomatic patients often present with a triad of overt symptoms synonymous with the infection (fever, lymphadenopathy, and pharyngitis) [7,17,18]. Other symptoms that may present include splenomegaly, malaise and palatal petechiae depending on how the virus affects the host [19–21]. A preliminary diagnosis of IM will be confirmed in the laboratory. Note, not all cases present clinically in the same way and typical and atypical cases may vary [5,22,23]. As IM is a viral illness the symptoms are commonly treated with rest, hydration, analgesia and antipyretics [7]; the use of steroids should only be given in cases where it is

CONTACT P Naughton pat_naughton@yahoo.com Department of Haematology, Mercy University Hospital, Grenville Place, Cork, Ireland
© 2021 British Journal of Biomedical Science

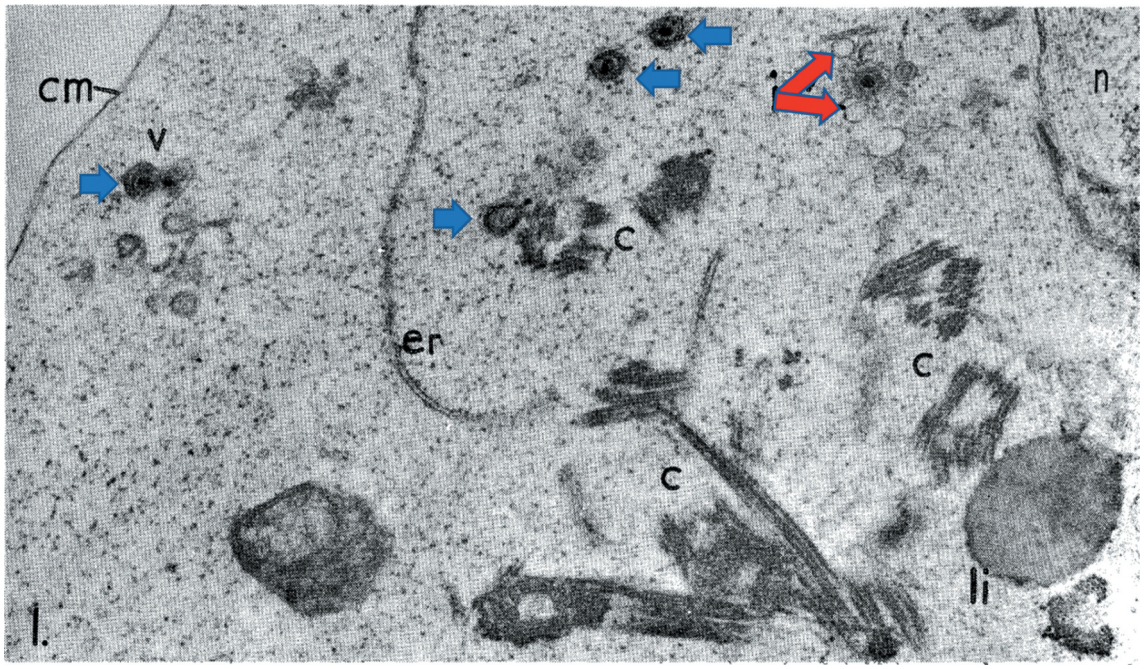

Figure 1. Electron micrograph (x 42,000) of part of a cultured lymphoblast derived from a sample of Burkitt's lymhoma [88]. (Blue arrows indicate mature EBV viruses; red arrows indicate immature EBV virus particles. The cell membrane of the lymphoblast is seen at the top left hand corner (cm), also observed in the image: crystals (c) a large lipid body (li), endoplasmic reticulum (er) and part of the cell nucleus (n).).

necessary to alleviate airway obstruction [7,24]. Inappropriate treatment for a bacterial tonsilitis can result in an ampicillin rash in a high percentage of cases [25,26]. This rash needs to be distinguished from an urticarial rash seen in cases of an allergic reaction to penicillin [25]. This differentiation would have an important bearing on any future course of antibiotic treatments for the patient. Treatment of IM with antiviral treatments such as aciclovir, valaciclovir and ganciclovir are still in their infancy and although initial results are promising further research along with patient trials are required [7]. Splenomegaly, on palpation or via ultrasound, is often a sign of IM, and splenic rupture, although rare (<1%), is a well-established complication of the disease and the most common cause of death [22,27]. As the incidence of this disease is predominantly encountered in adolescents and young adults, who are generally active and often involved in regular, sometimes strenuous exercise or team sports, it is ill-advised to return to sport or strenuous activity too soon after infection or to at least wait until any splenomegaly has resolved [22,28].

History of IM and its link to EBV

An early documented description of this distinct clinical syndrome was made by the German physician, Emil Pfeiffer in 1889. Pfeiffer used the German term 'Drüsenfieber', translated to glandular fever [29], to describe the clinical condition of the characteristic triad of symptoms of fever, lymphadenopathy (swollen lymph glands) particularly the cervical lymph glands of the neck (cervical lymphadenopathy) and pharyngitis [7,24,30–32]. Other less common symptoms of the disease include general malaise, fatigue and more uncommonly splenomegaly, swollen liver, and skin rash [9,19,24,33,34], although not all cases present clinically in the same way. Typical cases of IM present with at least two of the characteristic triad of symptoms but atypical cases can present differently and an appreciation of this is important when it comes to diagnostic investigation [5,11,23,30].

In 1932, Paul and Bunnell, while investigating for the presence of heterophile antibodies in several unrelated clinical conditions, detected strong titres of heterophile antibodies in four cases of acute IM [35]. Although underestimating its diagnostic importance at the time (thinking the discovery to be of little more than theoretical interest), it has subsequently led to the development of several diagnostic tests which aid in the detection of IM, some of which are still used today.

Henle, Henle and Diehl later showed unequivocally that a clear link between the disease and the virus existed and that EBV was most likely the etiologic agent for IM [36].

Category/Classification (Taxonomy)

EBV is a double-stranded DNA oncogenic virus classified under the order Herpesvirales as belonging to the Herpes family of viruses, Herpesviridae. These can be further classified into three subfamilies: α, β and γ (Table 1) [37]. Alpha-herpesvirinae include Human

herpesvirus 1 and 2 (HHV-1 and HHV-2) commonly referred to as herpes simplex virus types 1 and 2 (HSV-1, HSV-2) and Human herpes virus 3 (HHV-3) (more commonly known as the Varicella-zoster virus (VZV)) which cause herpes labialis/genitalis and chickenpox, respectively. Beta-herpesvirinae include Human cytomegalovirus (CMV) or Human herpesvirus-5 (HHV-5) and Roseolovirus (HHV-6, HHV-7) which can cause infectious mononucleosis like infections (IML) and Roseola Infantum, respectively [6,11,37]. Gamma-herpesvirinae include EBV (Human herpesvirus 4, HHV-4) the causative agent of IM which is also implicated in several human cancers including Burkitt's lymphoma, Hodgkin's and T-cell lymphomas and various gastric and nasopharyngeal carcinomas [9,38–41]. EBV is also associated with several autoimmune diseases including systemic lupus erythematosus and multiple sclerosis (MS) [34,42–46]. It is also implicated in associated cases of acquired hemophagocytic lymphohistiocytosis (HLH) [20,47]. The second virus in this subgroup is Kaposi's sarcoma-associated virus (KSHV) or more formally known as Human herpes virus 8 (HHV8) responsible for Kaposi's sarcoma [48].

Structure of EBV

EBV is a double stranded DNA virus [49]. Two types of EBV infect humans, EBV type1 (EBV-1) and EBV type 2 (EBV-2) [50], although there is a high level of similarity between both strains they differ primarily in the genetic sequences of their latent genes (EBNA2 and EBNA3); this gives rise to functional differences particularly in their B-cell transforming capacity [51–53]. Type 1 is able to convert B cells into lymphoblastoid cell lines more efficiently than type 2 [54]. EBV was sequenced in its entirety in 1984 [49]. Geographically, Type 1 strains are more prevalent worldwide although individuals can be infected with both strains [50].

Epidemiology of Primary EBV infection and IM

EBV is a ubiquitous human pathogen causing infection throughout the world in all human populations, infecting 95% of the world population at some point in life [19,21,36]. Seroepidemiological studies have demonstrated that 90–100% of adults over 60 years of age are seropositive for EBV [55,56]. IM is generally considered a first world disease where higher socioeconomic living standards within groups in certain populations results in delayed primary exposure to the virus [57,58]. In these cases, exposure usually occurs in adolescence, early adulthood, or less frequently older patients [11,58,59]. The prevalence of the disease in adults over 35 years of age is rare owing to the fact that a much higher percentage of this population will have acquired immunity [11]. It has been shown that the frequency of primary exposure to the virus later in life can lead to a higher proportion of clinically observed cases of IM [30]. Conversely, early exposure, which is more often the case in lower socio-economic groups and in general populations in the developing world, results in less observed cases of clinical IM [21,34,60]. Antibody levels to EBV are much higher in children from lower socioeconomic backgrounds and in developing countries when compared to children of a similar age in more affluent countries [11,36]. The disease might often go unnoticed or undiagnosed in children due to the absence of heterophile antibody responses and the persistent overreliance on these confirmatory tests to help diagnosis [11,24,30,36].

The population studies to date suggest that IM is a disease more commonly observed in adolescents, early adulthood and to a lesser degree older adults. However, it is unclear whether findings of group studies of IM cases to date are reflective of the actual case numbers or only the diagnosed cases bearing in mind that the appearance of the disease in younger patients (<10 years) may manifest differently and that the diagnosis of the disease in younger age groups may require separate criteria to unequivocally diagnose the disease. The heterophile antibody test is limited in its ability to diagnose heterophile positive IM cases only [11,31]. The disease may manifest differently in younger patients or atypical cases where a heterophile antibody response is often absent or significantly reduced [24,30].

Taking these factors into consideration a standard approach to IM investigation and laboratory testing regardless of patient age should be adopted to maximize the number of true positive cases detected [32]. This would encompass those subclinical or atypical cases of IM which often go either unreported or reported as equivocal [5].

Mode of infection with EBV

EBV is principally spread from an infected contagious person to another or others via the spread of saliva which contains the active virus. The usual route of spread is by oral contact [57,61,62]. Evidence has also supported the spread of the virus in infective individuals via blood transfusions and solid organ transplants, but the principal route of infection is through either direct or indirect spread of virus-laden saliva into the mouth [9,24,62]. The incubation period for naturally acquired IM (oral route) is 4 to 7 weeks from moment of contact [62]. The intermittent presence of EBV in saliva implies that there is not only active replication but also latent infection from a viral reservoir [57].

Pathogenesis

EBV has developed strategies which enable it to attach, penetrate and replicate within its target human host

cells. Over time a very definite host parasite relationship has developed; infection of the host, replication within the host, sequestering within the host cells and intermittent replication and re-infection of other host cells and ultimately the spread to other non-immune hosts [63]. In the normal acquired route of infection, the virus contained in the saliva of an infective host is spread into the buccal cavity of an uninfected individual where it travels through to the oropharyngeal region and Waldeyer's ring – tonsils and adenoids at the back of the mouth and throat [64]. The virus attaches, infects, and amplifies in the tonsillar epithelial cells before infecting naïve B cells in the underlying lymphoid tissue (the parenchyma) of the tonsil [9,64]. Infection of B-lymphocytes provides the virus a means of travelling throughout the body via the lymphoreticular system [46].This results in a systemic infection [9,64].

EBV infection occurs in the lymphoid system. The spleen is less often enlarged and delicate in atypical cases of IM but when it does occur there is a very real danger of splenic rupture which can on rare occasions be fatal; leading to advice to forgo strenuous physical sport and exercise when recovering from IM [7,22,65].

EBV, like other herpesviruses, has a productive lytic cycle and a dormant latent phase [9,63,66]. During the lytic cycle, regulatory proteins including early antigen are synthesized to allow the production of viral DNA (EBV-DNA), the virion structural proteins (viral capsid antigen, VCA) and membrane proteins. The lytic cycle terminates in the destruction of infected cells with the release of mature viral particles (virions) [63,67].

At the lytic stage of development, the infected B cells are most vulnerable to attack from cytotoxic T cells (CTLs) and the natural killer cells of the cell mediated immune response. These reactive cells are directed against the viral antigens of the lytic stage and act to control the growth of these transformed cells during primary infection, hence their tell-tale appearance during the acute phase of the infection [9].

EBV persists in the host B-cells without complete virus (virion) production in the latent stage of development thereby ensuring its survival and longevity within the host [68–70]. During the latent phase of development, the virus encodes and expresses a restricted number of viral proteins. These viral proteins activate B-cell proliferation transforming the cells into 'immortal' B cells (and by consequence the virus) which can replicate indefinitely. In order to do this EBV makes use of a series of distinct latent gene transcription programmes, which mimic a normal B cell response to antigen, to drive the differentiation of the newly infected B cells [63,66].

T-cells are also directed against antigens of the latent phase, but the response is insufficient to ensure their complete eradication and the virus can persist in the host for life with low or intermittent levels of virion production. EBV nuclear antigens (EBNAs) and certain latent membrane proteins are expressed in infected B-lymphocytes during the latent phase [9]. Eventually, the latently infected B cells enter the periphery or germinal centre, the site of viral persistence, as resting memory cells. These memory cells express only a very limited subset of the viral latent genes and hence are largely unaffected by the host's immune response [46,66].The latently infected memory cells circulate between the periphery and the lymphoid tissue [64]. These memory cells can, on occasion, circulate back to Waldeyer's ring at the oropharyngeal region at the back of the throat [24,46,71]. Here the memory B-cells can be triggered into reactivation culminating in rupture of the cell with the release of the infectious virus particles (virions) [11,64]. These virions, whilst all the while being the target of neutralizing antibody, can initiate intermittent new rounds of naïve B cell infection or re-infect the surrounding epithelium. This results in transient plaques of lytic epithelial infection that greatly amplifies the amount of infectious virus that is ultimately shed into the saliva for infectious spread to new hosts [46,57,66].

The virus has thus developed a sophisticated mechanism allowing its genome to persist in the host cells, to partition when the cells divide, and to switch from a latent state (with limited gene expression) to a fully replicative lytic state, in which mature viruses can be synthesized, to allow it to disseminate among other cells and ultimately other non-immune hosts [41,72].

Once the virus establishes in the human host, the immune system can never completely eradicate the virus [46]. The virus has developed an elaborate strategy of immune evasion that enables it to sequester and persist within the host well after the initial infection. In most immunocompetent individuals a host virus balance is maintained for life.

Cellular responses to EBV

Different viral proteins are expressed at different times during the life cycle of the virus as it attaches, inserts, develops, replicates, and spreads within the B lymphocytes of the host organism. This will in turn stimulate, in immunocompetent individuals, an appropriate and specific humoral and cell-mediated response to mitigate the effect of the invading virus and limit its damaging effects on the host.

Atypical lymphocytes

The classic clinical picture of IM is associated with the transient appearance of a distinct population of pleomorphic atypical lymphocytes observed during the acute clinical phase of the disease (Figures 2 and 3). This atypical lymphocyte population was the first characteristic biological marker associated with the disease.

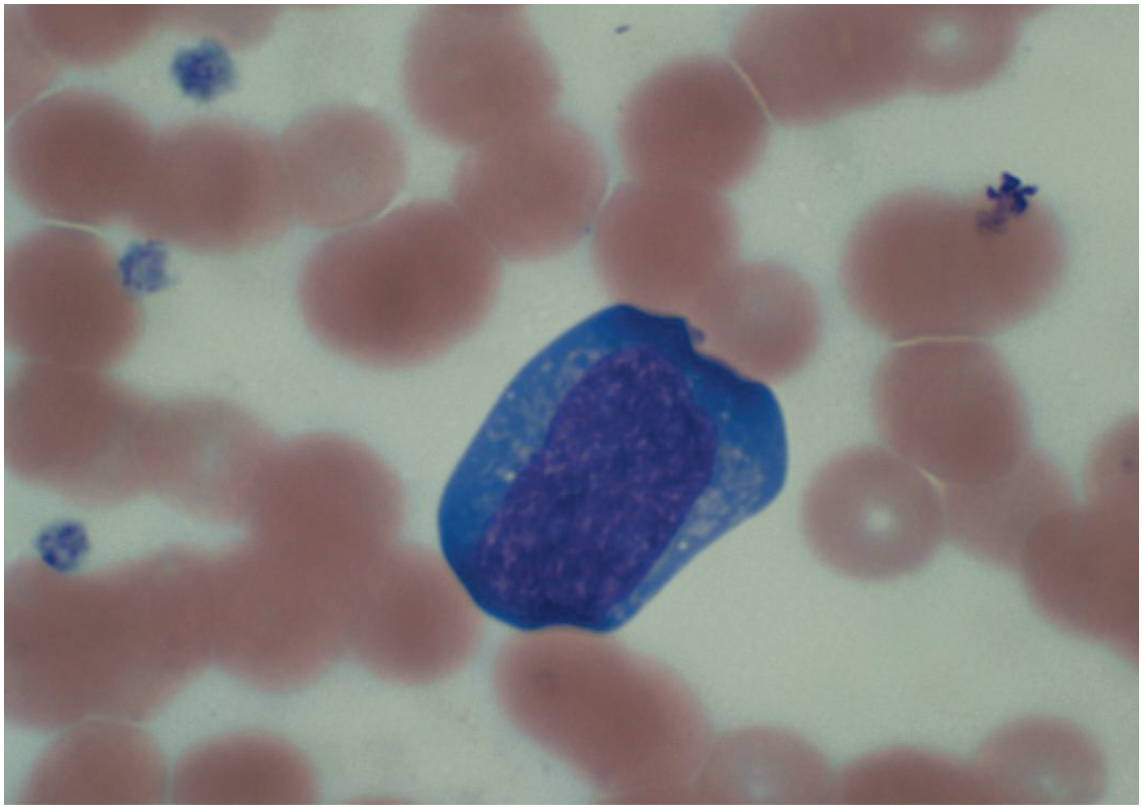

Figure 2. An atypical lymphocyte observed in the peripheral blood of a 24 year old female who presented as a case of heterophile negative acute IM.

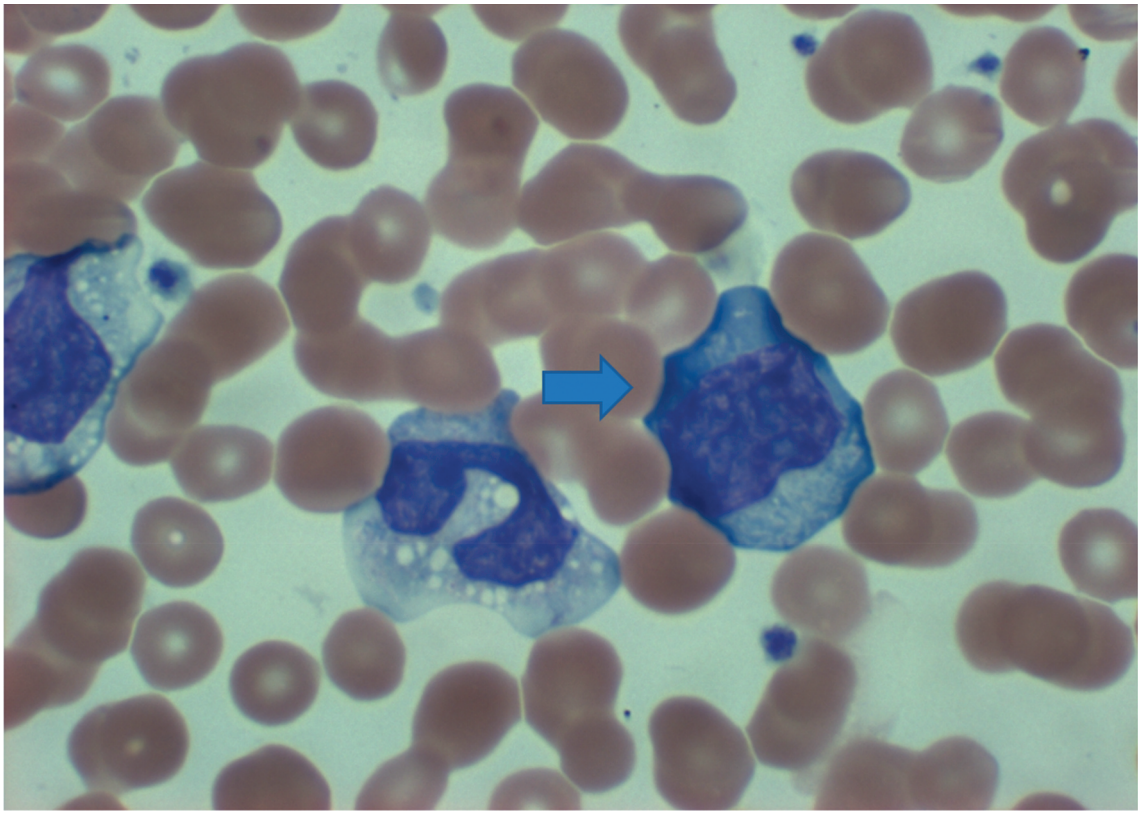

Figure 3. Reactive/atypical lymphocyte seen in an 18 year old female who presented as a heterophile positive case of acute IM.

But although suggestive, the appearance of these cells in isolation, was not diagnostic of the disease, as similar pleomorphic lymphocytes can be seen in other infectious states and malignant disorders [1,29]. However, these cells' customary appearance during the acute stage of IM and their subsequent gradual disappearance as the infection resolves has been found to be diagnostically useful [31]. Their transitory nature when first observed

Table 1. Classification of the order *Herpesvirales* [37,87].

Taxon	Name	Acronym	Common name
Order	*Herpesvirales*		
Family	*Herpesviridae*		
Subfamily	*Alpha-herpesvirinae*		
Genus	*Simplexvirus*		
	Human herpesvirus 1	HHV1	Herpes simplex virus type 1
	Human herpesvirus 2	HHV2	Herpes simplex virus type 2
Genus	*Varicellovirus*		
	Human herpesvirus 3	HHV3	Varicella-zoster virus
Subfamily	*Beta-herpesvirinae*		
Genus	*Cytomegalovirus*		
	Human herpesvirus 5	HHV5	Human cytomegalovirus
Genus	Roseolovirus		
	Human herpesvirus 6	HHV6	Human herpesvirus 6
	Human herpesvirus 7	HHV7	Humam herpesvirus 7
Subfamily	*Gammaherpesvirinae*		
Genus	Lymphocryptovirus		
	Human herpesvirus 4	HHV4	Epstein-Barr virus
Genus	Rhadinovirus		
	Human herpesvirus 8	HHV8	Kaposi's sarcoma-associated herpesvirus

was key in linking them with a then unknown infective agent.

Cytotoxic T lymphocytes

Given that EBV infects B-cells, fluoresence flow cytometry directed at CD molecules provided evidence that the circulating atypical cells are in fact reactive cytotoxic T-cells [5,9,32]. Given their nature, the detection and appearance of higher percentages of these cytotoxic T-cells/ atypical cells may be linked to more acute or active infection [32]. Further laboratory studies could determine what exact percentages are diagnostically significant and also ascertain whether their appearance present the same in both typical and atypical cases [5]. Alternative common names for these mononuclear cells are plasmacytoid or reactive lymphocytes, atypical lymphocytes, viral lymphocytes, Turk cells or Downey cells [29,73].

Serology

The host response to EBV includes a typical cell-mediated response by T-lymphocytes and a specific and non-specific humoral (antibody) response by the B-lymphocytes, including those B-lymphocytes which have already been infected by the virus. The humoral response of the host includes the production of antibodies to target viral antigens of both the lytic and the latent phases of viral development (Figure 4).

Heterophile antibodies

The EBV is capable of giving rise to both specific antibodies within the host and also non-specific (heterophile) antibodies generated as part of a general immune response to the disease. The chance discovery of increased and significant levels of these heterophile

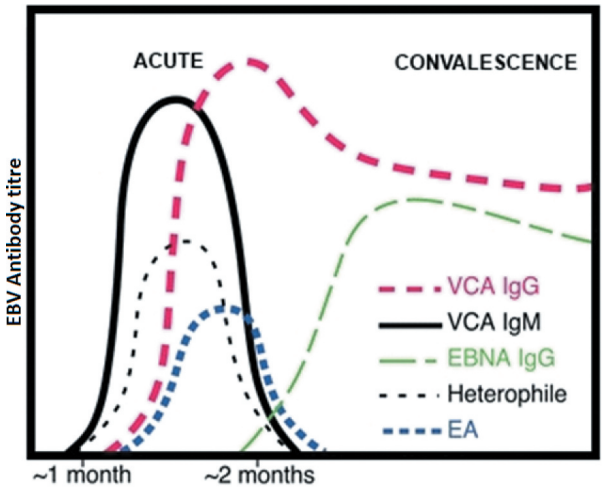

Figure 4. Time-related appearance of specific antibodies to EBV and non-specific Heterophile antibodies in cases of IM.

antibodies in a high proportion of acute cases of IM became the second commonly observed biological marker of the disease [35]. These non-specific antibodies are demonstrable by their ability to agglutinate animal erythrocytes including sheep (Paul Bunnell test) and horse (Monospot test) erythrocytes [74]. The Monospot test has proven to be more sensitive than the original Paul–Bunnell test in the detection of heterophile antibodies and has become the standard aid in the detection of IM in many laboratories and in primary care [74,75].

The presence of a high concentration of heterophile antibodies is a major feature of infectious mononucleosis [1,11]. These antibodies are produced as part of the direct immunologic response to infection and are brought about by the agent producing the disease. A positive Monospot test in the presence of a population of atypical lymphocytes in patients demonstrating distinct clinical symptoms is quite unequivocal in the diagnosis of IM; use of the test is limited though to typical heterophile positive cases of the disease.

Table 2. Summary of the common antibody screens specific for the Epstein-Barr virus and their clinical interpretation.

Possible results and interpretation for specific antibodies to EBV			
*VCA IgM	VCA IgG	^EBNA	Interpretation
–	–	–	No immunity to EBV
+	–	–	Active infectious mononucleosis infection
+	+	–	EBV serology suggestive of recent/active infection
+	+	+	Late primary infection (>8 weeks prior to sample date)
–	+	–	Past exposure (latent infection)
–	+	+	Previous EBV exposure
–	–	+	Past infection

*VCA = Viral capsid antigen; ^EBNA = Epstein-Barr virus nuclear antigen

Use of the Monospot test in heterophile negative cases of IM is of little value and can be misleading as incorrect interpretation could lead to the reporting of a false negative or equivocal result [76,77]. The persistence of high titres of heterophile antibodies in a percentage of post-acute IM cases could also lead to misinterpretation, particularly in the staging of the disease; there exists therefore the potential to erroneously diagnose a patient as an acute case long after the acute phase of the disease has passed [24]. This would have consequences if a secondary bacterial throat (streptococcal) infection occurred soon after the initial EBV infection and was not treated. Some of the principal advantages of the Monospot test as an aid to the diagnosis of IM is that it is quick, cost efficient, requires little training of staff and can, in certain cases, be conclusive, so mitigating the need for more follow-up tests.

EBV-specific antibodies

With the discovery that EBV was the specific etiologic agent responsible for IM came the identification and isolation of the specific antibodies produced by the immune system to target the pathogen and defend the host organism [11,36]. Following infection, the virus produces a number of distinct viral proteins which mark the different developmental stages of its cycle within the host. In response the body's immune system reacts by manufacturing different antibodies at different times which are directed against these foreign proteins [11]. The host's measured immune response is reflective of the virus's development, which has formed the basis for serological testing for the disease (Table 2).

Anti-EBV IgM Viral capsid antigen (Anti-EBV VCA IgM)

EBV specific IgM antibodies are detectable in the early acute phase of infection and tend to disappear within a few weeks of primary infection. These antibodies are directed against the outer coat (capsid) of the virus as it develops in the B-lymphocyte. Their presence, in the absence of later stage antibodies, is a very reliable early diagnostic marker for acute IM but it is not always routinely available in each site and can result in time delays when requested off site [5,9,11,34,67,78].

Anti-EBV IgG Viral capsid antigen (Anti-EBV VCA IgG)

The long-lasting IgG class antibodies appear a week or two after the appearance of the acute IgM class. These antibodies persist indefinitely and are an excellent marker for past infection and host immunity [9,24].

Anti-EBV nuclear antigen (Anti- EBNA IgG)

Antibodies against EBNA-1 develop slowly and are not normally detected until 8–12 weeks post onset of the illness. The presence of EBNA IgG antibodies rules out a recent primary EBV infection and hence is useful in the staging of the disease and for indicating past infections.

EBV early antigen (EBV EA IgG)

Testing for EA IgG in the diagnosis of acute EBV infection is limited by the fact that these antibodies are not always detected in individuals with acute IM and also by the fact that they can often remain at detectable levels in a high proportion of cases long after the primary infection [9,38,67,79].

Immunophenotyping

The development of modern immunophenotyping techniques has allowed for the study of the cell populations in acute IM. These studies have verified the presence of a large population of CD8+ cytotoxic-suppressor T cells (CD8 + T cells), believed to be part of the host cell-mediated immune response and responsible for the control of viral replication in the infected B-lymphocytes and the subsequent establishment of latency [4,18,80,81].

DNA analysis for EBV viral load

DNA analysis of the patients' plasma following PCR can detect the viral load in the patient. This is of particular value in immunocompromised individuals and in post-

transplant patients as primary or reactivated EBV infection in these patients can be associated with life threatening disorders such as post-transplant lymphoproliferative disorders [9,79]. Quantitative measurements of EBV DNA in the blood for the purposes of documenting primary EBV infections are unnecessary [24].

Supplementary tests

Liver function tests of alanine aminotransferase, gamma glutamyl transferase, and aspartate aminotransferase are usually raised during the acute phase of IM infection, implying a minor degree of hepatitis, but jaundice is rare [21,82–86].

Conclusion

Given the paucity of recent references on IM It is unsurprising that there is, as yet no international standard diagnostic algorithm for the laboratory investigation and testing for this disease. Despite the historical medical knowledge and research available on this disease and its causative agent the diagnosis is not always definitive. The correct diagnosis, when made, is often arrived at through an informed investigative process relying on knowledge of the disease, its aetiology, the causative agent, and its pathogenesis, being cognizant that not all cases are the same but that general guidelines can be followed. Acute markers, in particular the initial detection of a population of atypical lymphocytes can alert the investigator to the need for further analysis and the additional tests required to confirm a diagnosis.

A sharing of knowledge and a fluid multi-disciplinary approach to the investigative process would better serve all stakeholders and would help to shorten hospital stays, free up bed spaces, alleviate patient stress and allow appropriate patient management.

There is much value to be gained from studying the early, pioneering research into this disease and its causative agent. A contemporary retrospective and prospective analysis of laboratory data, in particular, could potentially guide a process to establish an international standard or algorithm for testing this disease. It is hoped that such an investigation would go some way to verifying or discounting some of the findings relating to this disease that can be currently found in the literature and potentially lead to a more standardized approach in the testing and diagnosis of this disease in the future. In so doing we would build on the solid foundations set by the early pioneers into this most interesting of human diseases and standardize a model of testing into the future.

Acknowledgments

The authors wish to thank Mr David Hegarty for IT expert guidance and Dr James O'Connor for scientific writing expert guidance, both of the Microbiology Department, MUH.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

B Lucey http://orcid.org/0000-0002-0872-202X

References

[1] Sprunt TP, Evans FA. Mononuclear leucocytosis in reaction to acute infections ("infectious mononucleosis"). Bull Johns Hopkins Hosp. 1920;31:410–417.
[2] Medović R, Igrutinović Z, Radojević-Marjanović R, et al. Clinical and laboratory differences between Epstein-Barr and cytomegalovirus infectious mononucleosis in children. Srp Arh Celok Lek. 2016;144:56–62.
[3] Klemola E, Von Essen R, Henle G, et al. Infectious-mononucleosis-like disease with negative heterophil agglutination test. Clinical features in relation to Epstein-Barr virus and cytomegalovirus antibodies. J Infect Dis. 1970;121:608–614.
[4] Hess RD. Routine Epstein-Barr virus diagnostics from the laboratory perspective: still challenging after 35 years. J Clin Microbiol. 2004;42:3381–3387.
[5] Taga K, Taga H, Tosato G. Diagnosis of atypical cases of infectious mononucleosis. Clinl Infect Dis. 2001;33:83–88.
[6] Cunha BA, Chawla K. Fever of unknown origin (FUO): CMV infectious mononucleosis or lymphoma? Eur J Clin Microbiol Infect Dis. 2018;37:1373–1376.
[7] Lennon P, Crotty M, Fenton JE. Infectious mononucleosis. Br Med J. 2015;350:h1825.
[8] Ishii T, Sasaki Y, Maeda T, et al. Clinical differentiation of infectious mononucleosis that is caused by Epstein-Barr virus or cytomegalovirus: a single-center case-control study in Japan. J Infect Chemother. 2019;25:431–436.
[9] Korsman SNJ, Van Zyl GU, Nutt L, et al. Epstein-Barr virus. In: Korsman SNJ, Van Zyl GU, Nutt L, et al., editors. Virology. Edinburgh: Churchill Livingstone; 2012. p. 58–59.
[10] Hurt C, Tammaro D. Diagnostic evaluation of mononucleosis-like illnesses. Am J Med. 2007;120:911.e1-8.
[11] Henle W, Henle GE, Horwitz CA. Epstein-barr virus specific diagnostic tests in infectous mononucleosis. Hum Pathol. 1974;5:551–565.
[12] Burkitt D. A sarcoma involving the jaws in African children. Br J Surg. 1958;46:218–223.
[13] Wright DH. Cytology and histochemistry of the Burkitt lymphoma. Br J Cancer. 1963;17:50–55.
[14] Burkitt D, O'Conor GT. Malignant lymphoma in African children. I. A clinical syndrome. Cancer. 1961;14:258–269.
[15] Burkitt D. A lymphoma syndrome in African children. Ann R Coll Surg Engl. 1962;30:211–219.
[16] Epstein MA, Achong BG. Fine structural organization of human lymphoblasts of a tissue culture strain (EB1)

from Burkitt's Lymphoma. J Natl Cancer Inst. 1965;34:241–253.
[17] Shubert S, Collee JG, Smith BJ. Infectious mononucleosis: a syndrome or a disease. Br Med J. 1954;1:671–675.
[18] Chatterjee B, Deng Y, Holler A, et al. CD8+ T cells retain protective functions despite sustained inhibitory receptor expression during Epstein-Barr virus infection in vivo. PLoS Pathog. 2019;15:e1007748–e.
[19] Womack J, Jimenez M. Common questions about infectious mononucleosis. Am Fam Physician. 2015;91:372–376.
[20] Kim WY, Montes-Mojarro IA, Fend F, et al. Epstein-Barr virus-associated T and NK-cell lymphoproliferative diseases. Front Pediatr. 2019;7:71.
[21] Xie J-L, Zhang Y-L, Zhou X-G. Infectious mononucleosis caused by EBV infection with clonal cytotoxic T cells in an infant patient. Int J Clin Exp Pathol. 2017;10:9802–9807.
[22] Baker CR, Kona S. Spontaneous splenic rupture in a patient with infectious mononucleosis. BMJ Case Rep. 2019;12:e230259.
[23] Louppides S, Kakoullis L, Parpas G, et al. Upper eyelid oedema in a patient with pharyngitis/exudative tonsillitis and malaise: hoagland sign in infectious mononucleosis. BMJ Case Rep. 2019;12:e233719.
[24] Dunmire SK, Verghese PS, Balfour HH Jr. Primary Epstein-Barr virus infection. J Clin Virol. 2018;102:84–92.
[25] Thompson DF, Ramos CL. Antibiotic-induced rash in patients with infectious mononucleosis. Ann Pharmacother. 2017;51:154–162.
[26] Chovel-Sella A, Ben Tov A, Lahav E, et al. Incidence of rash after amoxicillin treatment in children with infectious mononucleosis. Pediatrics. 2013;131:e1424–7.
[27] Asgari MM, Begos DG. Spontaneous splenic rupture in infectious mononucleosis: a review. Yale J Biol Med. 1997;70:175–182.
[28] Bartlett A, Williams R, Hilton M. Splenic rupture in infectious mononucleosis: a systematic review of published case reports. Injury. 2016;47:531–538.
[29] Downey HAL, McKinlay CA. Acute lymphadenosis compared with acute lymphatic leukemia. Arch Internal Med. 1923;32:82–112.
[30] García-Peris M, Jiménez Candel MI, Mañes Jiménez Y, et al. [Epstein-Barr virus primary infection in healthy children]. An Pediatr (Barc). 2019;90:376–385.
[31] Macsween KF, Higgins CD, McAulay KA, et al. Infectious mononucleosis in University Students in the United Kingdom: evaluation of the clinical features and consequences of the disease. Clinl Infect Dis. 2010;50:699–706.
[32] Ebell MH, Call M, Shinholser J, et al. Does This patient have infectious mononucleosis?: the rational clinical examination systematic review. Jama. 2016;315:1502–1509.
[33] Tattevin P, Le Tulzo Y, Minjolle S, et al. Increasing incidence of severe Epstein-Barr virus-related infectious mononucleosis: surveillance study. J Clin Microbiol. 2006;44:1873–1874.
[34] Auwaerter PG. Recent advances in the understanding of infectious mononucleosis: are prospects improved for treatment or control? Expert Rev Anti Infect Ther. 2006;4:1039–1049.
[35] Paul JR, Bunnell WW. The presence of heterophile antibodies in infectious mononucleosis. Am J Med Sci. 1932;183:91–104.
[36] Henle G, Henle W, Diehl V. Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. Proc Natl Acad Sci USA. 1968;59:94–101.
[37] Davison AJ, Eberle R, Ehlers B, et al. The order Herpesvirales. Arch Virol. 2009;154:171–177.
[38] Cui J, Yan W, Xu S, et al. Anti-Epstein-Barr virus antibodies in Beijing during 2013-2017: what we have found in the different patients. PLoS One. 2018;13:e0193171.
[39] Duca KA, Shapiro M, Delgado-Eckert E, et al. A virtual look at Epstein-Barr virus infection: biological interpretations. PLoS Pathog. 2007;3:1388–1400.
[40] Tsao SW, Tsang CM, Lo KW. Epstein-Barr virus infection and nasopharyngeal carcinoma. Philos Trans R Soc Lond B Biol Sci. 2017;372:1732.
[41] Xu M, Zhang WL, Zhu Q, et al. Genome-wide profiling of Epstein-Barr virus integration by targeted sequencing in Epstein-Barr virus associated malignancies. Theranostics. 2019;9:1115–1124.
[42] Cassaniti I, Cavagna L, Calarota SA, et al. Evaluation of EBV- and HCMV-specific T cell responses in Systemic Lupus Erythematosus (SLE) patients using a normalized enzyme-linked immunospot (ELISPOT) assay. J Immunol Res. 2019;2019:4236503.
[43] Gross AJ, Hochberg D, Rand WM, et al. EBV and systemic lupus erythematosus: a new perspective. J Immunol. 2005;174:6599–6607.
[44] Levin LI, Munger KL, O'Reilly EJ, et al. Primary infection with the Epstein-Barr virus and risk of multiple sclerosis. Ann Neurol. 2010;67:824–830.
[45] Handel AE, Williamson AJ, Disanto G, et al. An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis. PLoS One. 2010;5:9.
[46] Thorley-Lawson DA. EBV Persistence–Introducing the Virus. Curr Top Microbiol Immunol. 2015;390:151–209.
[47] Beutel K, Gross-Wieltsch U, Wiesel T, et al. Infection of T lymphocytes in Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis in children of non-Asian origin. Pediatr Blood Cancer. 2009;53:184–190.
[48] Machón C, Fàbrega-Ferrer M, Zhou D, et al. Atomic structure of the Epstein-Barr virus portal. Nat Commun. 2019;10:3891.
[49] Baer R, Bankier AT, Biggin MD, et al. DNA sequence and expression of the B95-8 Epstein-Barr virus genome. Nature. 1984;310:207–211.
[50] Young LS, Yap LF, Murray PG. Epstein-Barr virus: more than 50 years old and still providing surprises. Nat Rev Cancer. 2016;16:789–802.
[51] Coleman CB, Lang J, Sweet LA, et al. Epstein-Barr Virus Type 2 infects T cells and induces B cell lymphomagenesis in humanized mice. J Virol. 2018;92:e00813–18.
[52] Zanella L, Riquelme I, Buchegger K, et al. A reliable Epstein-Barr Virus classification based on phylogenomic and population analyses. Sci Rep. 2019;9:9829.
[53] Sample J, Young L, Martin B, et al. Epstein-Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B, and EBNA-3C genes. J Virol. 1990;64:4084–4092.
[54] Rickinson AB, Young LS, Rowe M. Influence of the Epstein-Barr virus nuclear antigen EBNA 2 on the growth phenotype of virus-transformed B cells. J Virol. 1987;61:1310–1317.
[55] Hornsleth A, Siggaard-Andersen J, Hjort L. Epidemiology of herpesvirus and respiratory virus infections. Part 1. Serologic findings. Geriatrics 1975;30:61–68.
[56] Sumaya CV, Henle W, Henle G, et al. Seroepidemiologic study of Epstein-Barr virus infections in a rural community. J Infect Dis. 1975;131:403–408.
[57] Niederman JC. Infectious mononucleosis: observations on transmission. Yale J Biol Med. 1982;55:259–264.
[58] Hallee TJ, Evans AS, Niederman JC, et al. Infectious mononucleosis at the United States Military Academy.

A prospective study of a single class over four years. Yale J Biol Med. 1974;47:182–195.
[59] Infectious mononucleosis and its relationship to EB virus antibody. A joint investigation by university health physicians and P.H.L.S. laboratories. Br Med J. 1971;4:643–646.
[60] Lennon P, Saunders J, Fenton JE. A longer stay for the kissing disease: epidemiology of bacterial tonsillitis and infectious mononucleosis over a 20-year period. J Laryngol Otol. 2013;127:187–191.
[61] Dunmire SK, Hogquist KA, Balfour HH. Infectious Mononucleosis. Curr Top Microbiol Immunol. 2015;390:211–240.
[62] Hoagland RJ. The incubation period of Infectious Mononucleosis. Am J Public Health Nations Health. 1964;54:1699–1705.
[63] Fitzsimmons L, Kelly GL. EBV and apoptosis: the viral master regulator of cell fate? Viruses. 2017;9:339.
[64] Laichalk LL, Hochberg D, Babcock GJ, et al. The dispersal of mucosal memory B cells: evidence from persistent EBV infection. Immunity. 2002;16:745–754.
[65] Becker JA, Smith JA. Return to play after infectious mononucleosis. Sports Health. 2014;6:232–238.
[66] Li C-W, Jheng B-R, Chen B-S. Investigating genetic-and-epigenetic networks, and the cellular mechanisms occurring in Epstein-Barr virus-infected human B lymphocytes via big data mining and genome-wide two-sided NGS data identification. PloS One. 2018;13:e0202537–e.
[67] De Paschale M, Clerici P. Serological diagnosis of Epstein-Barr virus infection: problems and solutions. World J Virol. 2012;1:31–43.
[68] Yetming KD, Lupey-Green LN, Biryukov S, et al. The BHLF1 Locus of Epstein-Barr virus contributes to viral latency and B-cell immortalization. J Virol. 2020;94:17.
[69] Pich D, Mrozek-Gorska P, Bouvet M, et al. First days in the life of naive human B lymphocytes infected with Epstein-Barr virus. mBio. 2019;10:5.
[70] Kenney SC, Mertz JE. Regulation of the latent-lytic switch in Epstein-Barr virus. Semin Cancer Biol. 2014;26:60–68.
[71] Hadinoto V, Shapiro M, Sun CC, et al. The dynamics of EBV shedding implicate a central role for epithelial cells in amplifying viral output. PLoS Pathog. 2009;5:e1000496.
[72] Tsurumi T, Fujita M, Kudoh A. Latent and lytic Epstein-Barr virus replication strategies. Rev Med Virol. 2005;15:3–15.
[73] Van Der Meer W, Van Gelder W, De Keijzer R, et al. The divergent morphological classification of variant lymphocytes in blood smears. J Clin Pathol. 2007;60:838–839.
[74] Seitanidis B. A comparison of the Monospot with the Paul-Bunnell test in infectious mononucleosis and other diseases. J Clin Pathol. 1969;22:321–323.
[75] Basson V, Sharp AA. Monospot: a differential slide test for infectious mononucleosis. J Clin Pathol. 1969;22:324–325.
[76] Burgio GR, Monafo V. Infectious mononucleosis fifty years after the discovery of the Paul-Bunnell test. Infection. 1983;11:1–5.
[77] Stokoe IH. Atypical mononucleosis and glandular fever. J R Coll Gen Pract. 1969;17:340–350.
[78] Hess RD. Routine Epstein-Barr virus diagnostics from the laboratory perspective: still challenging after 35 years. J Clin Microbiol. 2004;42:3381.
[79] Crowley A, Connell J, Schaffer K, et al. Is there diagnostic value in detection of immunoglobulin g antibodies to the Epstein-Barr virus early antigen? Biores Open Access. 2012;1:291–296.
[80] Tembhare P, Ramani M, Syed K, et al. Immunophenotypic profile in acute infectious mononucleosis mimicking malignant lymphoproliferative disorder: a case report and review of literature. Indian J Haem Blood Trans. 2010;26:118–121.
[81] Scherrenburg J, Piriou ERWAN, Nanlohy NM, et al. Detailed analysis of Epstein-Barr virus-specific CD4+ and CD8+ T cell responses during infectious mononucleosis. Clin Exp Immunol. 2008;153:231–239.
[82] Fugl A, Andersen CL. Epstein-Barr virus and its association with disease - a review of relevance to general practice. BMC Fam Pract. 2019;20:62.
[83] Petrova M, Muhtarova M, Nikolova M, et al. Chronic Epstein-Barr virus-related hepatitis in immunocompetent patients. World J Gastroenterol. 2006;12:5711–5716.
[84] Kang M-J, Kim T-H, Shim K-N, et al. Infectious mononucleosis hepatitis in young adults: two case reports. Korean J Intern Med. 2009;24:381–387.
[85] Herold J, Grimaldo F. Epstein-Barr virus-induced jaundice. Clin Pract Cases Emerg Med. 2020;4:69–71.
[86] Kofteridis DP, Koulentaki M, Valachis A, et al. Epstein Barr virus hepatitis. Eur J Intern Med. 2011;22:73–76.
[87] Davison AJ. Herpesvirus systematics. Vet Microbiol. 2010;143:52–69.
[88] Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet. 1964;1:702–703.