RANDOX

EDUCATIONAL GUIDE

Differentiating Viral from Bacterial Infections

Introduction

The distinction between viral and bacterial aetiology of infection is a difficult but crucial one to make. Although the mechanisms of infection and therapeutic options for viral and bacterial infections are considerably different, many patients are prescribed antibiotics before the source of the symptoms has been confirmed. Antibiotics prescribed in this manner; based on the clinician's experience and in the absence of an absolute diagnosis, are deemed empirical antibiotics and are one of the largest contributing factors to increasing antimicrobial resistance (AMR).

For example, in the US, approximately 90% of cases of acute bronchitis are of viral origin. However, as many as 80% of those treated received antibiotic therapy in an effort not to miss a bacterial infection[1]. AMR is considered one of the largest global health threats of our time. It is estimated that 4.95 million deaths were associated with AMR in 2019[2]. In addition to these complications, the misuse of antibiotics in children can have potentially life-threatening consequences including allergic reactions, neurological complications, and psychiatric disturbances[3].

To confirm the origin of a clinically significant infection, it is necessary to identify the infectious antigen. This can be achieved through diagnostic testing in the form of culture growth, molecular testing, or other antigen detection methods which are used in combination with presenting symptoms to confirm a diagnosis. Traditional differentiation between viral and bacterial infections takes the form of paired serology, which requires patients to visit a healthcare facility twice during a 2–4-week period. Many of these infections carry distressing symptoms; therefore, delaying treatment for this long is not practical. Novel, commercially available molecular techniques can reduce the time taken for a diagnosis dramatically, however, these techniques are often associated with high false positive rates and low specificity resulting in increased numbers of inappropriate antibiotic treatment.

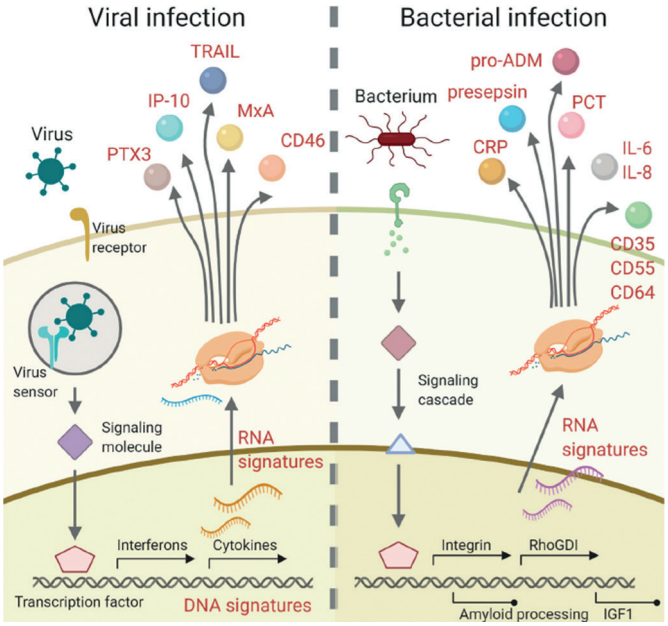

Figure 1. Illustration displaying the different infection pathways employed by viral and bacterial infections[3].

For some time, C-reactive protein (CRP) has been used as a biomarker for bacterial infection. This acute phase protein has been shown to be elevated in the presence of many bacterial infections within around 4-6 hours of infection, doubling in concentration every 8 hours, until the peak concentration is reached by around 50 hours[1]. However, while sensitive, CRP is not specific for bacterial infections, and concentrations are known to elevate during some viral and parasitic infections[4]. CRP quantification is also encumbered by its inability to identify viral, bacterial, or parasitic co-infections[1].

More recently, Mxyovirus resistance protein A (MxA) has been identified as a sensitive and specific marker for viral infections. MxA is an intracellular blood protein responsible for intrinsic resistance to a wide variety of viruses and is found to be elevated in most acute viral infections[1]. Although the mechanisms through which MxA act remain largely unknown, it shows strong potential as a novel biomarker for the differentiation between viral and bacterial infections.

The quantification of these biomarkers, used in concert, can provide a highly specific method for the differentiation of bacterial and viral infections. Herein, we discuss these biomarkers, what is known of their mechanisms, and their clinical significance.

C-reactive protein

CRP is a non-specific, acute phase protein, primarily induced by IL-6, which displays both pro-inflammatory and anti-inflammatory properties. It plays an essential part in identifying and removing pathogens and damaged cells. CRP achieves this by binding to key molecules involved in these processes such as phosphocholine, histones and chromatin, as well as the activation of the classical complement pathway and phagocytic cells[5]. As a result, CRP levels increase in response to inflammatory stimuli such as an infection.

These features of CRP make it an ideal biomarker for the identification of bacterial infection. However, CRP concentration is known to elevate in response to viral and parasitic pathogens such as Adenovirus, Influenza virus, Epstein-Barr virus, Malaria[1] and SARS-CoV-2[6], to name a few, eliminating its utility in the differentiation of these pathogens.

Normal serum CRP levels are 1-3mg/L and can range up to 500mg/L in response to severe inflammatory infections. CRP is currently used to identify bacterial infections; however, studies show inconsistent data regarding the sensitivity, specificity and AUROC (Area Under the Receiver Operating Characteristics)[4]. This disparity is partly due to the inconsistent cut-off values used in association with CRP concentration. Some studies claim a cut-off value of 20mg/L is ideal while others suggest a value of 50mg/L is more suitable and will help reduce false positive results[4].

Mxyovirus resistance protein A

Origin

MxA is transcribed by the interferon-stimulated gene (ISG), *mx1*, which was first identified as a factor conferring resistance to the Influenza A virus infection in mice[7]. Therefore, interferons (IFNs) are exclusively responsible for MxA synthesis. IFNs have no intrinsic anti-viral activity, but induce the expression of anti-viral molecules, such as MxA, in response to viral infection[7].

There are 3 types of IFN, Type I, II and III, which have distinct structures, properties, and activities. Type I and III display many similar functions, the key difference being the tissue they originate from. Type I IFNs, IFN-α, or leukocytes, and IFN-β, or fibroblasts, are commonly produced by all nucleated cells. In contrast, Type III IFNs, also known as IFN-λ, are almost exclusively produced by epithelial cells. Although there are fundamental differences between these types of IFN, they share similar biological and anti-viral activity[7].

Structure of MxA

Human MxA is a cytoplasmic protein closely associated with smooth endoplasmic reticulum (ER) which displays antiviral activity against positive- and double-stranded RNA viruses and some DNA viruses[8]. A comprehensive understanding of the mechanisms responsible for this viral interference remains elusive, however, new studies are beginning to shed light on the actions at work.

The 3D structure of MxA has not been completely resolved, with the tertiary structures of some of the MxA subunits proving difficult to determine. Nonetheless, the amino acid sequence responsible for these subunits, along with the known elements of the 3D structure has aided in clarifying the anti-viral mechanisms displayed by MxA[9]. A rendering of the known structure of MxA can be seen in Figure 2. MxA consists of a globular head which contains the GTPase or G domain and a stalk domain, attached via the bundle signalling element (BSE). The BSE is made up of 3 individual α-helices which fold the stalk back towards the G domain. Additionally, MxA sports 2 flexible loops deemed loop L2 and loop L4, and a N-terminal domain, none of which have resolved structures[9].

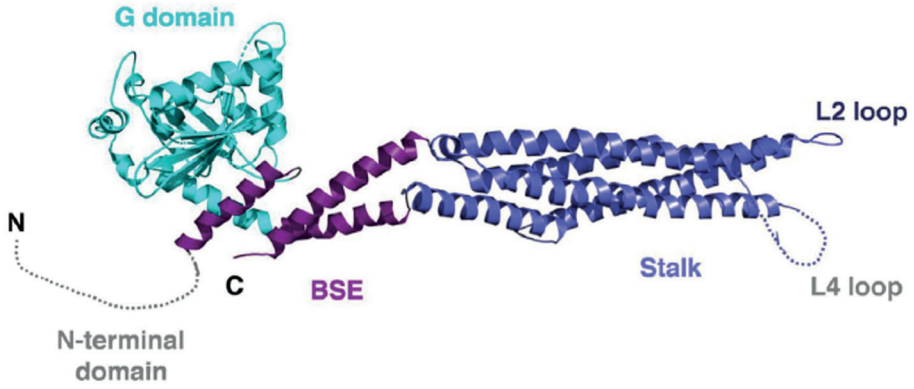

Figure 2. 3D structure of human MxA showing the known structures of the G domain, the bundle signalling element (BSE), the stalk and the L2 Loop subunits along with the as yet unidentified structures of the N-terminal domain and the L4 loop[9].

Structural and Anti-viral Activity of MxA

The full and true mechanisms in which MxA carries out its anti-viral activity are ambiguous. That said, accumulating evidence shows that the nucleoproteins of Orthomyxoviruses are sequestered by MxA resulting in the inhibition of viral amplification and infections[8].

For example, during infection with Influenza A virus (IAV), synthesis of viral mRNA is maintained within the MxA-positive cells, yet viral protein synthesis and viral amplification are almost completely inhibited. The genome of IAV is made of 8 RNA components known as the ribonucleoprotein complex (RNP) which is essential for viral infection. Once invasion of IAV has occurred, the RNPs are moved to the cytoplasm of the host, and then to the nucleus via active transport. The viral RNAs are then transcribed in the nucleus, the resulting mRNA is delivered to the cytoplasm for translation into the viral proteins. The new viral nucleocapsid protein (NP) is imported back to the nucleus where it is responsible for the synthesis of nascent virions (newly synthesised, immature virions). Therefore, the inhibition of viral RNP activity restricts the replication process of the virus and ultimately inhibits viral infection[8].

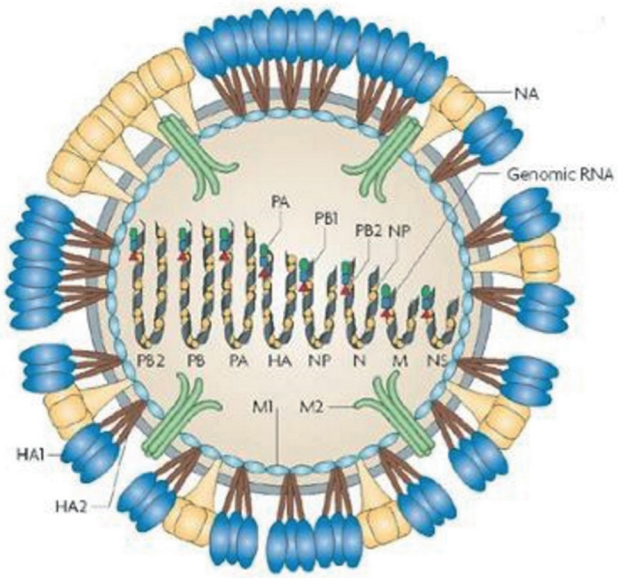

Figure 3. Illustration of the Influenza A virus[13].

Further studies show that MxA also binds to NPs and forms complexes within the cytoplasm which hinders its ability to enter the nucleus, resulting in an accumulation of these complexes near the site of the nucleus. These complexes are then removed by the smooth ER, preventing the synthesis and assembly of new viral components[8].

The human MxA protein is a member of the dynamin superfamily of multi-domain GTPases. These molecules can self-assemble and display GTPase activity. MxA binding with GTP facilitates the assembly of MxA by the ER and results in the assembly of MxA oligomers bound through the G domain. These oligomers organise in a ring-like structure around tubule liposomes. It has been proposed that these oligomers may also form around the tubular structures of viral RNPs, resulting in the anti-viral activity characteristic of MxA. Once formed, these ring-like structures are thought to prevent the assembly of enwrapped viral components and inhibit viral replication[7]. These MxA rings may also translocate viral nucleoproteins, resulting in their degradation. A representation of the MxA ring-like oligomer can be seen in Figure 4.

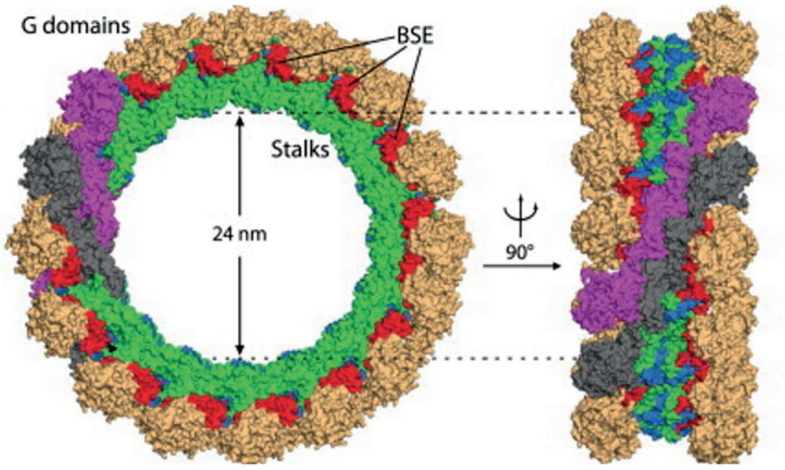

Figure 4. Rendering of MxA ring-like oligomer[14].

Finally, a study from 2023[9] investigated the N-terminal domain of the MxA protein and determined that it is essential for anti-viral activity. They showed that mutant MxA proteins which lack the N-terminal domain do not display anti-viral activity against IAV, Rhabdoviruses or Bunyaviruses and determined this was the result of a single crucial residue, namely Leucine 41. This research showed the highly conserved nature of this residue and confirmed the multi-mechanism nature of MxA anti-viral activity.

Clinical Evidence

Elevated CRP levels have been associated with bacterial infections for some time. A paper[4] compared studies published before and including 2019 that assessed the performance of CRP quantification in identifying bacterial infections. The authors report that 7 out of 7 studies showed significantly increased CRP concentration in patients with a confirmed diagnosis of bacterial infection compared with those without. The results of these studies reported AUROCs ranging from 0.62-0.91, displaying a lack of consistency across these studies. This inconsistency may be due to several factors, including but not limited to, different qualifying criteria for confirmed bacterial diagnosis and different study designs. Furthermore, this report revealed that 5 out of 5 studies found increased CRP concentrations in patients with malaria versus those without, displaying the lack of specificity of CRP quantification[4].

A study published in early 2023[10] measured MxA expression in a cohort of 61 adults with various bacterial, viral or co-infections using flow cytometry. This study also included patients receiving immunosuppressive therapy. The authors describe MxA expression in patients with viral infections (88.3 MFI) was significantly higher versus those who had bacterial infections (33.8 MFI), but not when compared with those identified to have a co-infection (53.1 MFI). Using a threshold of 62.2 MFI the authors report an AUROC for the discrimination of viral and bacterial infections of 0.9 with a sensitivity of 92.3% and specificity of 84.6%. Finally, this report revealed that immunosuppressive therapy had no significant effect on MxA expression in the presence of a viral infection or not.

Due to the additional adverse effects the overtreatment of antibiotics can have on children, studies in paediatric facilities have investigated the diagnostic power of CRP and MxA for the differentiation of bacterial and viral infections in children. The TREND study[11] reported that MxA levels were significantly higher in viral lower respiratory tract infections (LRTIs) than in bacterial LTRIs (777µg/L vs 145µg/L). Using a cut-off of 430 µg/L, this study displays an AUROC of 0.9 with a sensitivity and specificity of 72% and 100% respectively. Another study[12] investigated MxA levels to differentiate viral and bacterial infections in children using a lower cut-off value of 256 µg/L. Even with this lower cut-off value, this study shows promising results, with an AUROC of 0.81, a sensitivity of 74.4% and a specificity of 80%. The lower results displayed by this test may also be a result of a particularly robust study design in which children with definite, probably, and mixed origins of their symptoms were included.

Rapid Differentiation of Viral & Bacterial Respiratory Infections

MxA and CRP are useful biomarkers for the differentiation of viral and bacterial infections. To aid clinicians in achieving accurate diagnosis and to support antimicrobial stewardship, the Randox MxA & CRP kits, available on the VeraSTAT POC analyser.

VeraSTAT

The Randox VeraSTAT is a simple, accurate and portable POC device which delivers rapid results via the use of patented cathodic electrochemiluminescence technology (C-ECL). Through this technology, the target analyte in the patient sample reacts with the labelled antibody and captured antibody. After the reaction, unbound or excess labelled antibody is washed away and the labelled antibody complex is excited with electricity, with the electrochemiluminescence being measured and an accurate result produced.

The superiority of the VeraSTAT allows for users to overcome performance limitations of previous generation tests relating to sensitivity, accuracy, ease of use and cost efficiency. This, combined with a versatile test menu, means that the Randox VeraSTAT is built to outshine and enhance detection in any setting.

VeraSTAT MxA Kit

VeraSTAT MxA kit is an in vitro near-patient diagnostic test for the quantitative determination of Myxovirus resistance protein A from whole blood. The MxA Kit is used for detection of acute respiratory tract viral infections from symptomatic patients.

- Sample volume - 7µl
- Sample type – Whole Blood
- Measuring time – 11 minutes

VeraSTAT CRP Kit

VeraSTAT CRP kit is an in vitro near-patient diagnostic test for the quantitative determination of C-reactive protein from whole blood to assess the inflammatory status of the body.

- Sample volume - 5µl
- Sample type – Whole Blood
- Measuring time – 6 minutes

Used together, these tests can be used for the fast and accurate detection and differentiation of bacterial and viral infections from a small sample.

Figure 5. VeraSTAT POC Analyser.

Conclusions

The differentiation between viral and bacterial infections is a critical aspect of clinical practice, with significant implications for patient care and the global health threat of antimicrobial resistance. The use of biomarkers such as C-reactive protein (CRP) and Myxovirus resistance protein A (MxA) has shown promise in this differentiation, despite limitations. CRP, while sensitive, lacks specificity for bacterial infections, and its levels can elevate in response to viral and parasitic infections. On the other hand, MxA has been identified as a sensitive and specific marker for viral infections, with its mechanisms of action still being explored.

The combined use of these biomarkers can provide a highly specific method for differentiating bacterial and viral infections. The Randox VeraSTAT, a point-of-care device, offers rapid results for CRP and MxA, aiding clinicians in making accurate diagnoses and supporting antimicrobial stewardship. However, further research is necessary to optimize the use of these biomarkers and to explore other potential markers for infection differentiation.

The challenge of distinguishing between viral and bacterial infections underscores the importance of continued research in this area. As our understanding of the immune response to various pathogens evolves, so too will our ability to accurately diagnose and treat these infections, ultimately improving patient outcomes and combating the global threat of antimicrobial resistance.

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