Chapter 8
Assessing Biomarkers in Viral Infection

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Abstract Current biomarkers to assess the risk of complications of both acute and chronic viral infection are suboptimal. Prevalent viral infections like human immunodeficiency virus (HIV), hepatitis B and C virus, herpes viruses, and, more recently, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) may be associated with significant sequelae including the risk of cardiovascular disease, other end-organ diseases, and malignancies. This review considers some biomarkers which have been investigated in diagnosis and prognosis of key viral infections including inflammatory cytokines, markers of endothelial dysfunction and activation and coagulation, and the role that more conventional diagnostic markers, such as C-reactive protein and procalcitonin, can play in predicting these secondary complications, as markers of severity and to distinguish viral and bacterial infection. Although many of these are still only available in the research setting, these markers show promise for incorporation in diagnostic algorithms which may assist to predict adverse outcomes and to guide therapy.

Keywords Biomarker · Viral infection · C-reactive peptide · Procalcitonin · Inflammation · Coagulation · SARS-CoV-2

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P. C. Guest (ed.), *Application of Omic Techniques to Identify New Biomarkers and Drug Targets for COVID-19*, Advances in Experimental Medicine and Biology 1412, https://doi.org/10.1007/978-3-031-28012-2_8

1 Introduction

Chronic viral infections are associated with immune system activation and inflammation which may be responsible for a number of non-infectious disease complications. These can include the development of autoimmune manifestations including cytopenias, malignancy, and cardiovascular disease (CVD) [1, 2]. Recently, there has been increasing interest in predicting adverse outcomes from these infections resulting in the identification of biomarkers which may indicate the development of chronicity and assist with treatment decisions. With the most recent severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) pandemic, infection in some patients was prolonged resulting in the development of syndromes including long-COVID-19 (also known as post-COVID-19) and multisystem inflammatory disorder of childhood (MISC-C) [3, 4]. Inflammatory markers including interleukin-6 (IL-6), and more conventional markers like C-reactive protein (CRP) and procalcitonin (PCT) [5, 6], were offered as a component of the laboratory management of these patients although the interpretation of the results was not always straightforward. CRP and PCT are used routinely in severely ill patients, but a number of other inflammatory biomarkers, including endothelial markers and other cytokines, are not offered routinely. In some cases, inflammatory biomarkers have not been fully evaluated as prognostic markers although they are available as routine tests. D-dimers (or additional fibrin-degradation products) are a measure of fibrinolysis and are increased with bleeding and clotting [7], but this test has more recently been utilized to assess prognosis in patients with SARS-CoV-2 infections, independently of overt underlying coagulopathy or thrombosis [8]. The timing of sample collection, assay type, and the number of repeat analyses are poorly standardized, and this may reduce the utility of these markers in the clinical setting [6, 9]. Diagnostic and management guidelines have been issued by scientific bodies although these do not fully cover all clinical scenarios [10–12].

This review will focus on some chronic viral test cases including human immunodeficiency virus (HIV) infection, hepatitis B and C virus (HBV and HCV) infection, selected human herpes viruses, Kaposi-sarcoma herpesvirus (KSHV), and Epstein-Barr virus (EBV), as well as SARS-CoV-2.

2 Inflammatory Cytokines in Viral Infections

Cytokines are small protein molecules which are released by both immune effector cells and non-immune cells and which act to regulate immune function [13, 14]. A comprehensive discussion of all cytokines is outside the scope of this review, but recently, 3 cytokines, interleukin-6 (IL-6), IL-1, and tumor necrosis factor alpha (TNF-α) have been an area of focus in viral disease. These pleiotropic cytokines are the chief regulators of multiple inflammatory pathways [13, 15–17].

IL-6, TNF-α, and IL-1α are secreted by multiple cells including non-immune cells like epithelial and endothelial cells and some leukocytes [15, 18, 19]. IL-1β

production is more restricted to leukocytes (primarily myeloid cells) [15]. Production of these cytokines is upregulated in response to innate immune system activation through the binding of pathogen-associated molecular patterns (PAMPs) to highly conserved pattern-recognition receptors (PRRs) [13]. An important mediator of secretion of IL-1β specifically is the inflammasome, a complex of proteins containing PRRs, which recognize specific microbial patterns including the nucleotide oligomerization domain, leucine-rich repeat receptors (NLRs). The nitrogen permease regulator-like 3 (NLRP3) inflammasome activates caspase 1 which cleaves pro-IL1 into active components, IL-18 and IL-1β [20, 21]. TNF-α production is upregulated in response to IL-1β and toll-like receptor (TLR) activation through upregulation of TNF-α gene transcription. TNF-α is converted to a soluble form by the metalloproteinase TNF-α converting enzyme (TACE) [15]. Levels of IL-6, the principal member of the IL-6 family of cytokines, are low in healthy individuals but rise rapidly with inflammation [17]. IL-6 gene transcription is upregulated by nuclear factor kappa B (NFκB), nuclear factor IL-6 (NF-IL-6), and activation protein-1 among other pro-inflammatory signaling pathways, typically in response to PAMPs or danger-associated molecular patterns (DAMPs) [18]. Further secretion is stimulated by the action of the IL-6 amplifier which also positively influences secretion of other pro-inflammatory cytokines [18]. Elevated cytokine levels in chronic viral infections are attributed to a number of stimuli. In HIV infection, chronic activation has been linked to ongoing low-grade viral replication, presence of opportunistic infections, and microbial translocation [22]. Both EBV and KSHV promote inflammatory gene transcription, and KSHV produces viral cytokine homologs including viral IL-6 [23].

IL-1β, TNF-α, and IL-6 are crucial to pro-inflammatory responses [15, 18, 19]. All three are associated with monocyte and neutrophil recruitment and activation, dendritic cell maturation, increased endothelial permeability, fever, and pain. In response to these cytokines, there is release of acute phase proteins and hepcidin from the liver [24]. IL-1β, IL-6, and TNF-α (sometimes also classed as sT-helper 1 cytokines) promote a pro-inflammatory T-cell response and inhibit regulatory T-cell differentiation [25]. IL-6 specifically stimulates Th17 T-cell differentiation, in conjugation with transforming growth factor beta (TGF-β). It also has a non-redundant function in plasma cell differentiation and antibody secretion. IL-6 hypersecretion is also associated with increased platelet production and bone remodeling [17]. IL-1β favors Th17 differentiation in response to increased IL-6 levels by suppressing suppressor of cytokine signaling 3 (SOCS3) [15]. The IL-1 receptors are common entry sites for microorganisms, and expression and activity are therefore tightly regulated by mechanisms involving decoy receptors and proteolytic degradation [15]. As pro-inflammatory cytokines, IL-1β, TNF-α, and IL-6 promote an important antiviral and antibacterial response. However, under chronic infection and inflammation conditions, cytokine levels remain elevated, and this can become pathogenic [13, 16]. Therefore, these cytokines can have both beneficial and detrimental effects in viral infections [5, 6, 13, 26–61] (Table 8.1).

Table 8.1 Secretion and effects of inflammatory cytokines in selected viral infections

	Interleukin 1β (IL-1 β)	Interleukin-6 (IL-6)	Tumor necrosis factor-α (TNF- α)
Hepatitis B (HB) virus [13, 26–29]	Downregulation of secretion by HBe Antigen(Ag) and upregulation by HBcAg; increased levels associated with viral replication and disease complications including cirrhosis and HCC	Elevated levels inhibit viral entry and transcription; ongoing hypersecretion predicts mortality in acute on chronic liver failure and contributes to development of HCC through activation of the STAT3 pathway	Inhibition is associated with HBV reactivation; increased production also associated with liver fibrosis, hepatocyte apoptosis and pyroptosis
Human immunodeficiency virus (HIV) [30–32]	Augmentation of NLRP3 and IL-1B gene expression culminating in activation of the inflammasome in dendritic and related monocyte lineage cells with IL-1β hypersecretion	Elevated levels associated with lower CD4+ T-cell count and higher HIV viral load; strongly predictive of all-cause mortality and specifically HIV-associated CVD and non-AIDS defining malignancies	Increased secretion primarily by macrophages through action of viral proteins nef, tat and gp120; causes bystander immune cell apoptosis; elevated levels associated with increased mortality and disease progression
Hepatitis C (HC) virus [28, 33]	Upregulated in response to hypoxia during chronic inflammation; activates production of membrane metalloproteinase 9 with subsequent fibrosis; also linked to HCC and stimulation of an epithelial-mesenchymal transition	IL-6 polymorphisms linked to poorer outcomes with chronic HCV infection; may stimulate tumorigenesis through action on JAK-STAT pathway	Inhibition not conclusively linked to reactivation; putative role in hepatic fibrosis and hepatocyte pyroptosis
Epstein-Barr Virus (EBV) [32, 34–41]	Upregulated in response to viral proteins including LMP-1 although other viral proteins may inhibit secretion of IL-1 and downregulate its cognate receptors; increases are associated with pyroptosis but also with increased development of nasopharyngeal carcinoma and angiopathy in chronic infection; associated with development of chronic EBV disease and with HLH	Elevation predicts mortality in primary effusion lymphoma; biomarker for development of HL; independently associated with mortality in HL; Viral IL-6 associated with B-cell immortalization and hyperproliferation; prognostic marker and possible therapeutic target in EBV-associated HLH	High levels associated with elevation of early lytic proteins, including LMP-1, resulting in B-cell proliferation; elevated levels independently associated with EBV associated chronic fatigue syndrome and HLH

(continued)

Table 8.1 (continued)

	Interleukin 1β (IL-1 β)	Interleukin-6 (IL-6)	Tumor necrosis factor-α (TNF- α)
Kaposi-sarcoma herpesvirus (KSHV) [42–46]	IL-1α and/or IL1-β increased in response to vOX_2 glycoprotein b and other viral proteins; stimulates angiogenesis and abnormal cell proliferation and upregulates PD-1L to effect tumor cell escape; increased levels associated with tumorigenesis in KS, primary effusion lymphoma and multicentric Castleman's disease	Increased levels predictive of development of KSHV-associated malignancies including primary effusion lymphoma, KS and multicentric Castleman's disease; upregulates growth factors including Vascular Endothelial Growth Factor; high levels associated with KSHV-associated cytokine syndrome	Upregulated levels in response to KSHV glycoprotein b although other factors may inhibit secretion; elevated levels associated with viral reactivation, KS and B-cell lymphomagenesis; elevated levels may also be associated with decreased viral load
SARS-CoV-2 [5, 6, 47–61]	Levels of IL-1β, IL-6 and TNF-α are all raised in SARS-CoV-2 disease and have been predictive of severity, mortality and disease complications including neurological disease, severe viral pneumonia and development of lung fibrosis, multisystem inflammatory disorder of children, SARS-CoV-2 associated HSH and long COVID-19 syndrome; SARS-CoV2 cytokine release syndrome has been targeted with immunotherapies		

HCC hepatocellular carcinoma (HCC); *STAT3* signal transducer and activator of transcription 3; *NLPR3* nitrogen permease regulator-like 3; *CVD* cardiovascular disease; *nef* negative factor; *tat* transactivator of transcription; *GP* glycoprotein; *JAK-STAT* Janus kinase-signal transducer and activator of transcription; *LMP-1* latent membrane protein 1; *HLH* hemophagocytic lymphohistiocytosis; *PD-L1* programmed cell death Ligand-1; *KS* Kaposi sarcoma

3 Coagulation as a Biomarker of Viral Infection

Coagulation is a component of an innate immune response, and a procoagulant state is a feature of dysregulated inflammation [62]. Cardiovascular events including venous thromboembolic disease, myocardial infarction, cerebrovascular accidents, and thrombotic microangiopathies are a cause of virus-related morbidity and mortality [62]. Biomarkers may assess endothelial cell activation or clot formation or breakdown [7, 63]. Classically, disseminated intravascular coagulation (DIC) is a complication of severe sepsis and has been associated both with primary viral infection as a trigger and also with secondary conditions specifically cancer and bacterial or viral superinfection [64].

Both humoral and cellular effectors of coagulation have prognostic value in severe viral disease [14, 65, 66]. Thrombocytopenia is a key feature of ongoing microvascular thrombosis and chronic inflammation which can result in dysmegakaryopoiesis [67]. In addition, immune-mediated platelet destruction is associated with multiple viral diseases including hepatitis C [33], HIV [68], SARS-CoV-2

[69], and the herpes viruses [70]. On the other hand, platelet sequestration is associated with hypersplenism, which may complicate liver disease or may be a direct result of infection [71]. Increased platelet numbers may also be present specifically in response to elevated IL-6 [18]. Platelet activation is increased by multiple inflammatory mediators including the lipid mediators of inflammation contributing to pathological thrombosis [65].

Leukocytes can also contribute to infection-related thrombosis by interacting with both platelets and the endothelial surface. In HIV, there is upregulation of leukocyte expression of tissue factor which can activate factor VII stimulating the coagulation cascade [72]. Both platelets and monocytes upregulate expression of adhesion markers like P-selectin and its cognate ligand, P-selectin glycoprotein ligand [73]. Measurement of these markers, by immunophenotyping, can be an important adjunct in assessing risk and has been shown to correlate with CVD development and with other markers of viral severity [62]. Neutrophils, under inflammatory conditions, release neutrophil extravasation traps which also contribute to immunothrombosis by activating platelets and physically blocking the vascular lumen [74].

Chronic inflammation activates endothelial cells to a procoagulant and proinflammatory phenotype [62]. Endothelial dysfunction, a state of dysregulated contractility and endothelial cell activation, contributes to the development of CVD. Surrogate markers of endothelial dysfunction include the release of endothelial cell adhesion markers like intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and the procoagulant factors, factor VIII, and von Willebrand factor [62]. These factors can be pathogenic in thrombosis and have predictive value in critically ill patients.

Independent from CVD risk, coagulation system activation can predict severity in other complications of viral infection. For example, increased levels of ICAM-1 were found to be predictive of development of hepatocellular carcinoma (HCC) in chronic HBV and HCV infection [27], as well as decompensating cirrhosis [75]. Elevated levels of D-dimers are a strong predictor of mortality in HIV and specifically for CVD-related complications [76–78], and more recently, D-dimers have been used to prognosticate in severe SARS-CoV-2 infection [79]. Importantly, D-dimers show high negative predictive value in patients with suspected venous thromboembolic disease, and longitudinal measurement may indicate treatment adherence and clinical improvement [7].

4 Traditional Biomarkers of Severe Viral Disease

It can be difficult to distinguish bacterial from viral infections especially in the lower respiratory tract. Untreated bacterial infections can result in serious complications, while the use of antibiotics in inflammation or viral infections leads to the development of antibiotic resistance, increased costs, and possible unwanted side effects [80]. The most accurate way to diagnose these infections is by culture in the

case of bacterial infections, or serology for antibodies or antigens, or molecular tests. Culture results and ancillary test results are generally not available immediately, and there is a need for alternative approaches. Both CRP and PCT concentrations have been used to initiate and monitor the antibiotic use for lower respiratory tract infections [81].

These biomarkers also are elevated in people with inflammation resulting from causes other than infections such as trauma, autoimmune diseases, and metabolic disease [82]. Early studies during the COVID-19 pandemic suggested that these may be used as markers of disease severity.

4.1 CRP

CRP is an acute inflammatory protein discovered in 1930 by Tillet and Francis, while investigating the effects of sera of patients with pneumococcal pneumonia [83]. CRP binds to polysaccharides on microorganisms and activates C1q of the classical complement pathway [84]. CRP is synthesized primarily in hepatocytes, but is also produced in adipocytes, endothelial cells, lymphocytes, macrophages, and smooth muscle cells [85–87]. CRP is found in two forms: a pentameric form which can then dissociate to form monomers. These two forms of CRP play distinct roles in the inflammatory process [88]. Monomeric CRP is involved in the innate immune system by activation of the complement cascade and stimulation of both angiogenesis and thrombosis, whereas pentameric CRP is mostly released to the circulation after an inflammatory stimulus and recognizes phosphocholine on bacterial cells and damaged host cells [89].

CRP triggers C1q activation in the complement pathway leading to the opsonization of pathogens. It can also stimulate cell-mediated pathways via complement activation and by binding Fc receptors of IgG [90]. CRP increases within 4–6 h, in response to injury, infection, and inflammation, and peaks at about 36 h. In general inflammation, CRP levels can rise beyond 10 mg/L [89]. Lower concentrations of CRP, in the range of 0.01 to <10 mg/L (high sensitivity CRP or hsCRP), are associated with low grades of systemic inflammation. Low grade systemic inflammation is associated with elevated hsCRP levels, and use of this biomarker to detect atherosclerotic vascular disease has been intensely investigated through observational studies and clinical trials over the past two decades. On the basis of evidence that has accrued, hsCRP measurement has been integrated into the Reynolds risk scoring system to predict cardiovascular risk [91]. It is used at concentrations of <1 mg/L, 1–3 mg/L, and >3 mg/L to classify individuals as low, intermediate, or high risk for CVD, respectively [24].

Sequential CRP levels are a sensitive and specific biomarker to improve the differential diagnosis between acute bacterial and viral infections, although this may be less accurate in severe viral disease cases and with prolonged inflammation [92]. CRP is raised in patients with severe SARS-CoV-2 [93, 94] and can predict mortality [49, 95] especially in patients aged 60 years and older [96]. CRP levels show a

downward trend in survivors and tend to increase prior to death in non-survivors [97]. CRP kinetics in SARS-CoV-2-infected patients admitted to intensive care units were similar to those seen in bacterial sepsis with an initial rise followed by a decline during recovery, although levels are typically higher in patients with bacterial sepsis compared to patients with severe COVID-19 disease [98]. Mortality in patients with SARS-CoV-2 is higher in patients with comorbidities such as type II diabetes mellitus and preexisting CVD [99]. SARS-CoV-2 infection itself can cause cardiovascular damage and impaired glucose control. While biomarkers such as high sensitivity Troponin and pro brain natriuretic peptide (proBNP) are better markers of CVD, CRP is also elevated signifying the underlying inflammatory process [100]. CRP measurement can be an important ancillary test in these patients as it may directly damage cardiac tissue by activating complement, reducing nitric oxide (NO) release and CRP-mediated inhibition of angiogenesis, and stimulating endothelial cell apoptosis [101].

Elevated CRP levels have been associated with poorer outcomes in other viral infections such as SARS-related pneumonia, Middle East respiratory syndrome (MERS) infection, and H7N9 influenza. High levels of CRP were consistently seen with severe disease outcomes in H1N1 influenza patients [102–105]. Elevated CRP is also predictive of mortality in HIV particularly from CVD, and the levels of this biomarker are further elevated in patients with co-infection with other viruses like HCV [106]. The IL-6 expressed by KSHV also stimulates CRP secretion, and high CRP levels are a feature of a cytokine storm in a number of different viral diseases [14]. Taken together, these findings indicate that CRP is elevated in several viral infections and, therefore, cannot be used to differentiate between them.

4.2 PCT

PCT is a glycoprotein precursor of calcitonin released by the thyroid parafollicular cells. In healthy subjects, calcitonin is released, but in the presence of an inflammatory stimulus, particularly bacterial endotoxin or pro-inflammatory cytokines, there is increased calcitonin gene expression, and PCT mRNA is synthesized. This leads to release of PCT from all parenchymal tissues. PCT is a useful biomarker to differentiate between bacterial and viral infections as a concentration $\geq$0.5 μg/L is suggestive of a possible bacterial infection [107]. PCT may be used in the early diagnosis of bacterial pneumonias and to guide initiation of antibiotic therapy [108].

Although relatively specific for bacterial infections, serum PCT levels also correlate with disease severity and thus cannot reliably distinguish between bacterial and nonbacterial infections in the setting of critical illness, particularly in cases of severe influenza and SARS-CoV-2 infection [6, 52]. However, the value of PCT as a prognostic marker in SARS-CoV-2 is unclear. Meta-analyses have shown that those patients with severe disease had higher PCT levels compared to those with

non-severe disease [6, 109], although this was inconsistent with some studies failing to find a significant difference [51]. The reasons for these discrepancies may be attributed to variable cut-offs, patient ages, or other factors impacting PCT release. PCT release is inhibited by interferon (INF)-γ, for example, and levels of this cytokine may differ in different patient populations or with different administered therapies. Since INF-γ is a key antiviral cytokine, this could explain the differences in PCT level in viral and bacterial infection [110]. However, all three pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) stimulate parenchymal PCT production. PCT levels are typically normal in uncomplicated viral infections [111] but may rise with severe complications including, for example, the development of hemophagocytic lymphohistiocytosis (HLH) [9] or the development of secondary bacterial infection in patients with severe viral disease including H1N1 influenza [112]. In general, however, PCT appears to be a more specific marker of bacterial sepsis than CRP, albeit with some limitations. This has prompted a search for more specific markers or combinations of markers that can be used reliably to differentiate bacterial and viral infections.

One potential biomarker for distinguishing between bacterial and viral infections is myxovirus resistance protein A (MxA), an IFN-inducible protein with antiviral activity. MxA has been investigated for use as a biomarker because of its rapid induction in acute, symptomatic viral infections and low levels in bacterial infections and in healthy individuals [113–115]. Clinical studies, mostly involving children, suggest that MxA is selectively increased in viral infections and have the potential to rapidly distinguish viral and bacterial disease [116, 117]. It has been used in the emergency department setting to distinguish SARS-CoV-2 from bacterial and non-infectious causes of respiratory disease [118].

5 Conclusions and Future Perspectives

Viral infections cause significant morbidity and mortality. Host- and virus-specific factors can determine patient outcomes in both acute and chronic infection although these outcomes cannot always be predicted in clinical settings with the current biomarkers available, as demonstrated during the COVID-19 pandemic. In this review, we considered some of the biomarkers that are used in the clinical setting and in research to monitor viral infections. These biomarkers may predict the development of end-organ diseases including CVD and malignancies and contribute to acute viral immune escape or control, or they may indicate severe complications including HLH and cytokine release syndromes. Combinations of these markers can also help to distinguish between bacterial and viral infection which is critical for effective antimicrobial stewardship. Into the future, standardization of biomarker panels, validation of new markers, and appropriate age-specific, disease-specific reference ranges will assist to make these biomarkers more clinically relevant.

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