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Recent updates of interferon-derived myxovirus resistance protein A as a biomarker for acute viral infection

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Abstract

Background Antibiotic resistance (AMR) remains a global public health threat with a high burden in sub-Saharan countries. The overuse of antimicrobials in the clinical setting is the main factor for the spread of antibiotic resistance. Diagnostic uncertainty in differentiating between bacterial and viral infections is the major contributor to antimicrobial overuse. The available biomarkers lack specificity in guiding clinicians to make antibiotic decisions and only estimate bacterial infection.

Main body Myxovirus resistance (Mx) proteins are a type of interferon (IFN)-inducible protein that belongs to the dynamin superfamily of large guanine triphosphates (GTPases) involved in broad antiviral responses. Myxovirus resistance protein A (MxA) is a host-derived biomarker with antiviral properties against various viruses. It is induced by IFN I and IFN III as part of the innate immune response. Its basal level is < 15 ng/ml and elevated levels are detectable 1–2 h after IFN induction and remain detectable in serum up to 10 days after viral infection. Increased levels in the blood are associated with viral infection and remain low during bacterial infections. This biomarker showed promising performance in diagnosing undifferentiated febrile patients with respiratory tract infections. In this review, we discuss the role of Mx proteins, specifically MxA, in diagnosing acute viral infections, including how they are induced and their potential as diagnostic tools.

Methods A comprehensive electronic search was conducted in Scopus and Medline (using the PubMed interface) regarding myxovirus resistance protein A as a biomarker for acute viral infection. In the search strategy, English language was used without date restriction. Manual search was also performed when appropriate.

Conclusions Elevated MxA combined with other biomarkers, such as CRP and PCT, is a promising tool for identifying patients with viral infections. Therefore, incorporating MxA in the existing point of care formats help to improve the antibiotic stewardship programs and future randomized controlled trials are recommended to evaluate its utility in medical practice.

Keywords Acute virus infection, Biomarker, Interferon, Myxovirus resistance protein A

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Introduction

Antimicrobial resistance (AMR) has been declared a global public health threat to humans in the twenty-first century [1]. It has been estimated that the annual death toll associated with AMR is approximately 4.9 million, with figures showing that it disproportionately affects sub-Saharan nations [2]. The number of annual deaths is projected to increase to 10 million by 2050 [3]. Although multiple factors contribute to the spread of drug resistance, the overuse of antimicrobials in the clinical setting [4] and in animals [5] has made a substantial contribution.

Diagnostic uncertainty in the differentiation of viral and bacterial pathogens is the major contributor to the overuse of antimicrobial agents [6]. Differentiating between these pathogens early in life in children and adults [7, 8] remains challenging and clinicians inappropriately administer antibiotics due to concerns about missing bacterial infections [9].

In this context, physicians could misuse antibiotics without knowing the exact etiology of the infection, which leads to unnecessary adverse outcomes, such as antimicrobial resistance, secondary infections, and readmission, leading to increased health costs. However, as an alternative to alleviate these problems, point-of-care diagnostic tests have recently been used that might detect states such as the systemic inflammatory response associated with bacterial or viral infection and these tests could be used to guide clinicians in prescribing antibiotics in the clinical setting [10].

Host-derived markers such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and white blood cell (WBC) count lack the ability to discriminate between bacterial and viral infections [9, 11]. Procalcitonin (PCT), WBC and CRP are standard laboratory tests indicating the severity of the disease [12]. Compared to CRP and WBC, PCT has better specificity in identifying bacterial causes [13]. However, it is not sufficiently accurate to discriminate bacterial infections from nonbacterial infections [14]. Moreover, the biomarkers currently available only predict bacterial infection [9]. Pathogen-specific diagnosis of viral infection to rule out the cause of acute fever can be impractical, relative to cultivable bacterial infections, especially in resource-limited nations. Although polymerase chain reaction (PCR) tests are being used for different viral pathogens, low detection rates and nonpathogenic respiratory tract colonization are limitations to this method [15]. This highlights the need to investigate effective, rapid, and cost-effective diagnostic tools to identify those in need of antibiotics. Myxovirus resistance protein A (MxA) is a promising candidate biomarker that can be utilized as a viral infection marker when integrated to point-of-care formats. In this review, we discuss how MxA is induced and its role in the antiviral response. This biomarker could be relevant for discriminating viral infections from bacterial infections, thereby playing a prominent role in antimicrobial stewardship.

Methods

We used a comprehensive electronic search in Scopus and Medline (using the PubMed interface) by combining keyword terms for "MxA" or "myxovirus resistance protein A", "acute virus infection", "biomarker", "type I interferon" and "type III interferon" within all search fields in English language without date restrictions. A manual search was also conducted. All the articles were screened initially to select relevant articles.

Overview of myxovirus resistance proteins

Myxovirus resistance (Mx) proteins are interferon (IFN)-inducible proteins that belong to the dynamin superfamily of high molecular weight guanine triphosphatases (GTPases) involved in a broad antiviral response [16, 17]. IFNs are soluble components of the host defence mechanism that induce IFN-stimulated gene expression. They have been established as important mediator during viral infection [18, 19]. Notably, Mx proteins are key components produced as a result of interferon type I and III action [20–22] and are accepted as a general biomarker during acute viral infection [7, 23].

The interferon-responsive genes *Mx1* and *Mx2* were originally identified as resistance factors against lethal influenza A virus (IAV) in mice [19]. The two *Mx* genes are closely linked and are located on the long arm of chromosome 21 in humans [24]. To date, hundreds of interferon-stimulated gene (ISG) networks have been identified as the cornerstone of the innate immune system [25, 26]. Similar repertoires of ISGs are induced by IFN I and IFN III signaling leading to gene transcription. The expression of human *Mx1* and *Mx2*, produces antiviral proteins (MxA and MxB), respectively, which are among the well-known ISG products [19].

The two Mx proteins share 63% similarity in their amino acid sequence [27]. Structural resolution revealed that Mx proteins share a common domain structure. The three-dimensional structure of both GTPases is composed of a globular N-terminal GTPase (G) domain, bundle signaling element (BSE), and C-terminal stalk [28, 29]. The G domain and the stalk are connected by the BSE [18, 19]. The two Mx proteins have distinct amino acid sequences within their N-terminal regions of the BSE region and L4 loops of the stalk [19]. The inter- and intramolecular interactions between the stalk and BSE enable the protein to undergo oligomerization and mediate antiviral activity [28]. The antiviral target specificity

is directly determined by the L4 loop, which is located in the stalk domain of MxA [22] and the N-terminal domain of MxB [30].

Subcellular localization determines the antiviral activity of Mx proteins [31, 32]. Human MxA is located in the cytoplasm associated with the endoplasmic reticulum [33, 34] and is known to restrict the replication cycle of various viruses that have both cytoplasmic and nuclear replication phases [7, 21, 35]. MxB is located around nuclear pores [31] and has been identified as an inhibitory factor against a limited number of viruses such as human immunodeficiency virus-1 (HIV-1) [36] and herpesviruses [37], thereby blocking the nuclear import of viral nucleic acids [38, 39]. Recent studies have shown that MxB can also inhibit hepatitis C virus (HCV) [40] and hepatitis B virus (HBV) [41]. MxA has a broader antiviral spectrum than MxB and hence a more promising candidate biomarker. Therefore, we aimed to determine the role of MxA as a general biomarker for discriminating viral from bacterial infections.

Although human MxA was initially identified as an antiviral agent against influenza A virus [7], further studies revealed that MxA can restrict the production of a wide range of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) viruses early during their replication cycle by interacting with the viral nucleocapsid structure [21, 35]. Nevertheless, the MxA protein is unable to inhibit the replication of numerous viruses, such as HIV, where the antiviral activity against HIV involves MxB [42]. This is due to the differences in the interaction domain of the Mx proteins with different target viral components. The effector domain of MxB is located in the N-terminal region and the GTPase region [43, 44], while that of MxA is located in the loop-4 region [22]. Despite the lack of recent evidence, it has been observed that the level of MxA remains elevated during chronic HBV and HCV infections [45, 46]. On the other hand, the MxA level is downregulated during gamma-herpes virus infection [47, 48].

There is growing evidence showing that MxA could be a sensitive and specific biomarker of viral infection in different populations and clinical settings [49, 50]. Its diagnostic value has been investigated in the differentiation of viral from bacterial etiologies in acute febrile illness [7], respiratory tract infections [6, 50, 51], and COVID-19 [52, 53]. As the product of IFN-I activity, MxA is used to evaluate IFN-β and IFN-α bioactivity. In predicting the response to IFN-β in patients with multiple sclerosis (MS) the performance of MxA was best in indicating the presence of neutralizing antibodies (Nabs) to IFN-β [54]. Similarly, MxA expression was used to evaluate the bioavailability of IFN-α in HCV patients treated with IFN-α [55].

Production of myxovirus resistance proteins

Following the recognition of ligands such as viral nucleic acids and viral proteins by innate immune receptors, type I and type III IFN responses are activated as a major first line of defence [56, 57]. IFNs induce the expression of a large number of interferon-stimulated (ISG) genes during viral infections [19]. An antiviral state can be induced by three unique interferons called type I, type II, and type III IFNs [58], all of which induce ISG expression [19, 59]. MxA belongs to these ISGs and evidence suggests that its production in mammals is exclusively regulated by type I and III IFN but not by type II IFN [18, 60]. Even though the regulatory role of type II IFN in the MxA system is not observed in mammals, evidence indicates that IFN II regulates MxA in fish after viral infection of the kidney and spleen [61].

The binding of IFN I and IFN III to their cognate receptors can activate similar signaling pathways and the transcription of genes [62]. Both type I and type III IFNs can initiate the Janus kinase (JAK1) and signal transducer and activator of T cells (STAT) signaling pathways, resulting in the induction of ISGs. Type I interferon has a receptor, that is expressed in all nucleated cells. It is composed of interferon-α receptor 1 (IFNAR1) and interferon-α receptor 2 (IFNAR2) heterodimers [63]. Type III IFN (IFN-λ) has receptors that are expressed predominantly by epithelial cells [19]. Signals of type III IFNs are transduced via IFN λ receptor 1 (INFλR1) paired with the IL-10R2 subunit. This receptor complex is utilized by all type III IFN members (IFN-λ1, IFN-λ2, IFN-λ3, and IFN-λ4); in contrast, all type I IFNs utilize receptor subunits composed of IFNAR1 and IFNAR2 [64].

In both type I and type III IFN ligands, upon interaction with their cognate receptors, activation of receptor-associated JAK1 and tyrosine kinase (TYK2) phosphorylate STAT1 and STAT2 [19, 64]. IFNAR2 has a high affinity for type I IFN and IFNλR1 has a high affinity for type III IFN; both are associated with JAK1 which initiates IFN-mediated intracellular signaling [58]. Activated JAK1 is then able to phosphorylate STAT1 and STAT2 tyrosine motifs, thereby resulting in the heterodimerization of STAT1 and STAT2, which interact via their Src homology 2 (SH2) domains [65], and recruit interferon regulatory factor-9 (IRF-9) to form a trimeric transcription factor complex called IFN-stimulated gene factor-3 (ISGF-3) [18, 66]. This transcription factor then enters the nucleus and binds to interferon-stimulated response elements (ISREs) on the interferon-stimulated gene promoter region [65].

In the transcription factor complex, the DNA binding domain of IRF9 and STAT1 is recognized by a consensus region at the promoter of interferon-stimulated genes, but the STAT2 DNA binding domain interacts with the

nonconsensus region of the ISRE [67]. Interferon signaling ultimately produces antiviral proteins such as 2′, 5′-oligoadenylate synthetase, RNAseL, dsRNA-activated protein kinase, and Mx proteins all of which can mediate the antiviral activity of interferons [68]. Human cells defective in IFN production and STAT1 gene expression did not produce MxA upon infection with influenza virus. On the other hand, these cells produced interferon-stimulated gene 56 (ISG56) indicating that ISG56 can be produced in the absence of IFN. This indicates that in contrast to that of other ISGs, the expression of the *Mx* gene is not directly triggered by viruses and is instead reliant on type I and III IFN signaling [60]. This indicates that the production of Mx protein is the best marker of IFN action. A summary of the production of Myxovirus resistance protein A is shown in Fig. 1.

Antiviral mechanisms of myxovirus resistance protein A

Viral infection triggers the innate defence mechanism to prevent infection from spreading to tissues and removing virally infected cells [69]. Double-stranded (ds) RNA-dependent protein kinase R (PKR), 2'–5'oligoadenylate synthetase (OAS), viperin, tetherin [39], and Mx are IFN-induced proteins with broad antiviral activity. Among these, the most effective antiviral molecules are PKR, OAS and MxA [70].

The antiviral effector MxA inhibits the translation of viral proteins and or interferes with the nuclear translocation of newly produced viral proteins [71]. The loop (L4) domain of the MxA protein exhibits antiviral properties that determine its antiviral specificity [72]. In humans, cytoplasmic MxA recognizes the viral nucleoprotein and binds to its effector domain (L4) [22] to prevent its nuclear localization [73], thereby preventing viral gene transcription. In the cytoplasm of infected cells, MxA-nucleoprotein aggregates, which is considered an effect of antiviral activity [73]. This interaction results in the blocking of the nuclear entry of incoming viral nucleic acids [74].

Utility of myxovirus resistance protein A for differentiating viral from bacterial infection

Even though the production of type I and III IFNs is induced by the presence of viral infection [62], these IFNs cannot be used as diagnostic markers due to their short half-life in serum [7]. Conversely, MxA has an average

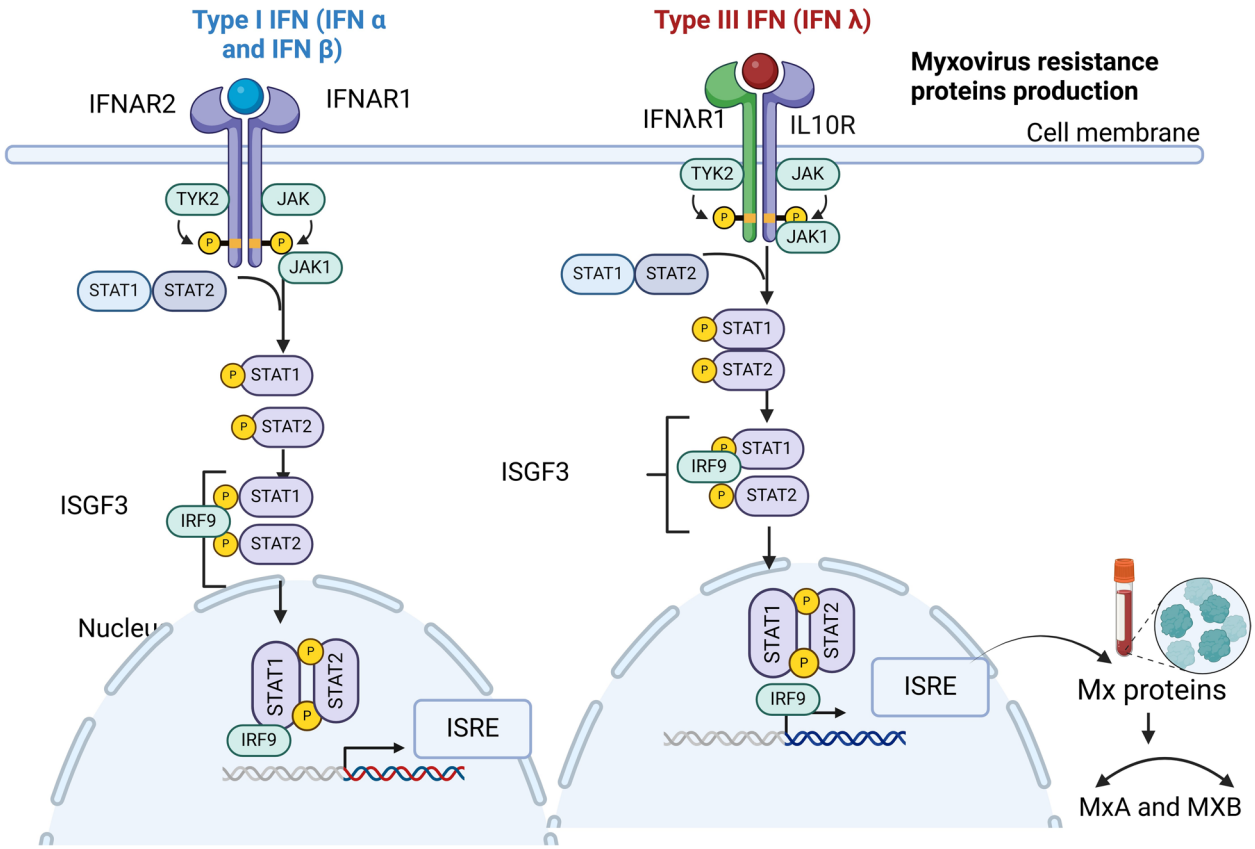

Fig. 1 Production of Myxovirus resistance protein. As shown in the figure, MxA protein can be induced by the type I IFN and type III IFN signaling pathways. The figure was created with https://app.biorender.com

half-life of 2–3 days in serum at very low basal concentrations (< 15 ng/ml), and a short induction time (1–2 h) [75]. It remains detectable in serum for up to 10 days after viral infection [76]. Moreover, the MxA gene does not respond to cytokines, including IL-1 and TNF-α and its expression is not elevated in healthy individuals [77].

More importantly, the level of MxA in children infected with a single virus was similar to that in children coinfected with multiple viruses [23], indicating the presence of viral infection even if it does not indicate a particular virus [75, 78]. Similarly, the level of MxA in adults depends on the severity of disease rather than the type of virus [49] and a similar result was found during the COVID-19 pandemic [21]. The level of MxA detected during viral–bacterial coinfections was similar to that detected during pure viral infections [17, 49–51] indicating an important limitation of using MxA alone in the differentiation of viral infections from bacterial infections. Studies have supported the combined use of MxA and other biomarkers, such as CRP and PCT, as invaluable tools for improving the accuracy and determination of the type of infection [19, 79]. Combining MxA with other viral markers such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), interferon-gamma inducible protein 10 (IP-10) as well as bacterial markers such as CRP, PCT, interleukin 6 (IL-6), and neutrophil gelatinase-associated lipocalin (NGAL) was promising in distinguishing bacterial from viral infection in febrile children with MxA/TRAIL pair demonstrating strong performance [80].

Some intracellular bacteria including *Mycobacterium tuberculosis, Chlamydia trachomatis, Listeria monocytogenes, Salmonella enterica serovar Typhimurium, Francisella tularensis, Legionella pneumophila, Brucella abortus* and *Coxiella burnetii* upregulate type I IFN production [81–83]. Specifically, Mycobacterial infections induce the production of MxA in macrophages [84]. However, the dynamics of MxA expression during other intracellular bacterial infections have not been well characterized [82, 83]. This underscores the need for careful interpretation of increased MxA levels, as withholding antibiotics without appropriate bacterial screening could lead to adverse outcomes [49].

A notable limitation is observed during Mycobacterium tuberculosis infections, where elevated levels of MxA, CRP, and PCT are documented [84, 85]. Although MxA is primarily known as a biomarker for acute viral infection, its utility extends to other contexts such as autoimmune diseases [86, 87]. Furthermore, MxA has been identified as a positive indicator in conditions like breast cancer [88] and pulmonary fibrosis [89]. These associations must be considered when using MxA as a diagnostic tool in clinical settings.

Use of the MxA protein as a diagnostic biomarker in a clinical setting and unanswered questions

Although comprehensive data for the real-world evaluation of MxA are not yet available, different studies have shown promising results as summarized in Table 1. In this table, algorithm-based laboratory comparators were used with expert adjudication. While MxA was used together with CRP in the context of FebriDx, the performance data provided in Table 1 are specific for MxA, showing its diagnostic ability as an independent biomarker.

The majority of published MxA data are from FebriDx MxA point-of-care test (POCT) devices (Table 1). The FebriDx test (Rapid Pathogen Screening, Sarasota, FL, USA) is a POCT that detects MxA alone, CRP alone or both qualitatively [90] at a predefined cutoff and lower limit of detection 40 ng/ml for MxA and 20 ng/ml for CRP [91, 92]. The test method utilizes anti-MxA and anti-CRP monoclonal antibodies to detect MxA and CRP, respectively [93]. FebriDx is approved by the FDA (U.S. Food and Drug Administration) and receives a CE (European Commission) mark for compliance with European Union (EU) regulations [94, 95]. It has shown promising results in diagnosing febrile patients with respiratory tract infections [96, 97]. The diagnostic value of the assay was better in those who exhibited fever during admission than in those who reported fever within 72 h [97]. These results suggest that higher MxA levels are more strongly associated with febrile infections than with afebrile respiratory tract infections [23]. The specificity of FebriDx MxA increased when the MxA ELISA was included in the reference algorithm to detect viral infections in patients with no identified pathogen by the reference method [96, 97]. On the other hand, some MxA-positive patients were negative according to PCR and were later found to have radiological changes of COVID-19. The authors reported that 7 patients who were positive for FebriDx MxA became negative by PCR and showed classic radiological COVID-19 features [98]. This is supported by the heterogeneous RT-PCR results obtained during the COVID-19 pandemic [99], indicating that MxA can complement PCR tests during the diagnosis of viral infections.

More importantly, FebriDx MxA is specific to pathogenic viruses, whereas PCR may detect nonpathogenic viruses without host immune response. When PCR is used as a reference test, it may compromise the performance of FebriDx, as some PCR-positive commensals were reported as false negative by FebriDx [12]. This is supported by the evidence that the production of MxA in asymptomatic controls with respiratory virus colonization is significantly lower than that in symptomatic patients with viral infection [23]. Furthermore, the MxA

Table 1 Diagnostic accuracy of myxovirus resistance protein A for differentiating between viral and bacterial infections

Study design	Year, Country	Type of cases/ patients	Cases tested	Viral infections	Reference test	MxA method	Study purpose	Sensitivity value, 95%CI	Specificity value, 95% CI	Refs.
Prospective	January to February 2014, USA	Adults and children with febrile URTI	205	53	✓Algorithm which includes PCR, procalcitonin, white blood cell count, IgM for EBV and bandemia	FebriDx	Evaluation	87, 75–95	83, 76–89	[96]
Prospective	November 2015 to July 2016, USA	Children and adults with acute upper respiratory tract infections	220	124	✓Algorithm which includes PCR, procalcitonin, white blood cell count, lymphocytosis and bandemia with physician over read	FebriDx	Evaluation	90, 83–94	76, 66–84	[97]
Prospective	March 2019 to November 2020	Adults with respiratory tract infection	244	39	✓PCR	FebriDx	Validation	49, 36–63	94, 90–97	[12]
Prospective	August 2020 to January 2021, Italy	Adults with acute respiratory tract infection due to COVID-19	200	136	✓PCR	FebriDx	Evaluation	97.8, 93.7–99.5	95.3, 86.9–99.0	[52]
Prospective	March to April 2020, UK	Adults hospitalized with COVID-19	478	222	✓Composite of PCR and Radiology	FebriDx	Evaluation	83.8, 78.3–88.4	93.3, 89.5–96.0	[100]
Prospective	March to April 2020, UK	Adults hospitalized with COVID-19	251	118	✓PCR	FebriDx	Evaluation	93, 87–97	86, 79–92	[98]
Retrospective	March 2022 to March 2023, UK	Adult patients suspected for multiple respiratory viral infection	5426	1709	✓PCR	FebriDx	Evaluation	91.1, 85.5–94.6	86.8, 80.2–91.5	[94]
Prospective	March 2020 to April 2020	Adults suspected for COVID-19	49	34	✓PCR or antibodies to SARS-CoV-2	FebriDx	Evaluation	100, 87.4–100	100, 71.7–100	[93]
Prospective	October 2019 to April 2021, USA	Adults and children suspected of ARI	520	296	✓Algorithm which includes PCR, procalcitonin, CBC & IgM for EBV	FebriDx	Evaluation	70.3, 64.8–75.2	88.0, 82.8–91.8	[6]
Prospective	April 2004 to March 2007, France	Febrile Children 0–16 years, suspected for viral and bacterial infection	553	133	✓PCR, Immunochromatography, Immunofluorescence test	EIA	Validation	97.4, 90.9–99.6	100, 91.9–100	[7]
Prospective	November 2017to December 2019, Sweden	Children 1–59 months with LRTI	326	242	✓PCR	EIA	Evaluation	93, 78–99	100, 51–100	[50]

Table 1 (continued)

Study design	Year, Country	Type of cases/ patients	Cases tested	Viral infections	Reference test	MxA method	Study purpose	Sensitivity value, 95%CI	Specificity value, 95% CI	Refs.
Prospective	March to June 2021, Netherlands	Adults with suspected COVID-19 infection	100	77	✓PCR	EIA	Evaluation	94, 85–98	91, 72–99	[53]
Prospective	December 2018 to June 2019, Austria	Adults suspected of bacterial and viral infection	61	26	✓PCR	BD FACS flow cytometry	Evaluation	92.3,75.9–98.6	84.6, 66.5–93.9	[49]
Prospective	July 2022 to February 2023, Greece	Adults, with respiratory tract infections	537	181	✓PCR	AFIAS-10 quantitative MxA	Validation	79.7, 74.5–85	80, 67.6–92.4	[51]
Prospective	May 2020 to September 2022	Febrile children	228	112	✓EIA	Lab master LUCIA	Evaluation	92	91	[112]

AFIAS-10 automatic fluorescence immunoassay system-10, *COVID-19* coronavirus disease 2019, *EBV* Epstein–Barr virus, *EIA* enzyme immunoassay, *FACS* fluorescent activated cell sorting, *IgM* immunoglobulin M, *LRTI* lower respiratory tract infection, *PCR* polymerase chain reaction, *Ref.* reference, *UK* United Kingdom, *URTI* upper respiratory tract infection, *USA* United States of America

levels were similar in PCR-positive children who tested positive for commensals and in PCR-negative asymptomatic controls [50]. This evidence confirmed that MxA is specific for symptomatic viral infections, unlike PCR. When the PCR protocol is specific for COVID-19, FebriDx MxA demonstrated comparable sensitivity to PCR and better sensitivity than lateral flow tests that detect the COVID-19 antigen [100]. Because of its high sensitivity and high negative predictive value across different COVID-19 prevalences, the assay method is recommended for use as a triage tool in patients suspected of having COVID-19 [94, 98].

The diagnostic accuracy of FebriDx MxA was limited among acute dengue virus cases in Ethiopia [91]. This could be because dengue can block type I IFN-mediated signaling and hence prevent the production of MxA [101]. In addition, certain viruses such as the measles virus [102], and herpes simplex virus type 1 (HSV-1) [103] are known to disrupt IFN I and IFN III production. Therefore, identifying such viruses, especially in endemic areas will be important if MxA is used for viral diagnosis.

To date, controversial results have been generated on the importance of MxA in diagnosing viral infection among immunocompromised patients. MxA production in immunocompromised patients was reduced and the sensitivity of viral identification using FebriDx MxA was significantly lower than that in immunocompetent patients [12]. In contrast, high doses of immunosuppressive drugs did not affect MxA detection among COVID-19 patients according to the same FebriDx method [52]. The expression of monocyte MxA in immunocompromised and immunocompetent viral patients was also similar [49]. An enzyme immunoassay-based study indicated that immunosuppressive medication or immunodeficiency does not affect MxA production in SARS-CoV-2 infected patients [53]. While the majority of the results indicated the effectiveness of MxA in diagnosing viral infection in immunocompromised patients, additional research is necessary.

The quantitative measurement of MxA enables the development of optimal cutoff points. The enzyme Immunoassay (EIA) method was used to evaluate different MxA thresholds for differentiating between viral and bacterial infections, and 200 ng/ml was reported to be the best combination of sensitivity and specificity in children [7]. The same method was used to determine the optimal cutoff value for MxA in adults with confirmed COVID-19 which was found to be 252 ng/ml [53]. Likewise, the optimal cutoff was found to be 430 ng/ml in children using the EIA [50].

Recently, the Automatic Fluorescence Immunoassay System-10 by Boditech Med (Chuncheon, Republic of Korea) was used to detect MxA in confirmed viral infections even when the MxA concentration was less than 15 ng/ml. Instead of using a predefined cutoff, these authors recommend that the MxA/CRP ratio, with a cutoff value of 2, better differentiates between viral and bacterial infections and showed a specificity of 75.7% (95% CI 67.9–82.2) and negative predictive value of 90.4% (95% CI 83.5–94.5) in predicting viral infection [51]. BD FACS flow cytometry (BD Life Sciences, New Jersey, USA), revealed that the MxA/CRP ratio was significantly greater in viral infections than in bacterial infections and bacterial–viral coinfections. The authors showed that the MxA/CRP ratio at a cutoff value of 4.7 has improved diagnostic accuracy and differentiates between viral and bacterial infections with a sensitivity of 84.6% (95% CI 66.5–93.9) and specificity of 100% (95% CI 87.1–100) [49]. Furthermore, MxA/CRP ratio at a threshold of 20 predicts viral infection in children with a sensitivity and specificity of 91.5% (95% CI 79.6–97.6) and 84.9% (95% CI 68.1–94.8), respectively [7]. This test method addresses the gap observed in qualitative test methods, as viral cases such as COVID-19 increase both MxA and CRP [93, 104], and the qualitative methods may produce false-positive bacterial results for the increased CRP levels.

Compared to quantitative assays, qualitative assays have several limitations. The qualitative FebriDx assay produces results as positive and negative without informing the disease severity/stage. However, increased concentrations of MxA could predict the severity of the disease/stage as was the case for COVID-19 patients [21]. Moreover, acute viral cases were detected with a level of less than 15 ng/ml [51], which could not be detected by a qualitative assay. Furthermore, the median basal level of MxA in children is significantly greater than that in adults (110 ng/ml and 10 ng/ml [23]; hence, FebriDx testing in children may produce false-positive results. The median basal level of MxA in children < 2 years of age is greater (160 ng/ml) than that above this age [17]. Therefore, the test method is not indicated for the diagnosis of viral infection in population aged < 2 years [90].

The qualitative (FebriDx) test method, however, is advantageous because it is an easy-to-use, rapid, handheld, single-use device and does not require additional equipment to produce results [90, 92]. FebriDx is intended to be used only in professional healthcare settings, and the manufacturer recommends retesting invalid results using a new test device [105]. The test is safe and requires a small amount of blood (5 µl) from the fingertip via a noninvasive procedure [106]. There is no adverse event related to testing and the time to result is short (10 min) [90, 92]. The assay method is intended to diagnose patients older than 1 year of age suspected of having viral and bacterial acute respiratory tract

Table 2 Diagnostic performance of different biomarkers for diagnosing viral infections

Biomarker	Target	Patients	Diagnosis	Patients tested	Viral infections	Study purpose	Reference standard	Sensitivity value, 95%CI	Specificity value, 95%CI	AUC value, 95% CI	Refs.
TRAIL	Febrile	Adult	Viral from bacterial	54	13	Evaluation	✓PCR ✓Viral culture	NR	NR	0.72, 0.56–0.88	[113]
IP-10										0.72, 0.59–0.86	
IFI27	RTI	Adult & children	Influenza from bacteria	402	96	Validation	✓PCR	80	90	0.9, 0.83–0.95	[114]
	SARS-CoV-2	Adults	Presymptomatic SARS-CoV-2	96	41	Validation	✓PCR	84, 70–93	95, 85–98	0.95, 0.91–0.99	[115]
IFI44L	SARS-CoV-2	Adults	Presymptomatic SARS-CoV-2	96	41	Validation	✓PCR	55, 40–70	95, 85–98	0.9, 0.84–0.96	[115]
	Febrile	Children	Virus from bacteria	25	11	Validation	✓PCR ✓Direct immunofluorescence	90.29	92.8	0.94, 85.3–100	[116]
IFN-α	Febrile	Children	Virus from bacteria	101	46	Evaluation	✓Expert panel	NR	NR	0.93, 0.87–0.98	[79]

AUC area under the curve, *IFN-α* interferon alpha, *IFI44L* interferon-induced protein 44-like, *IFI27* interferon alpha inducible protein 27, *IP-10* interferon gamma-induced protein 10, *NR* not reported, *PCR* polymerase chain reaction, *Ref.* reference, *RTI* respiratory tract infection, *SARS-CoV-2* severe acute respiratory syndrome coronavirus 2, *TRAIL* TNF-related apoptosis-inducing ligand

infections [105]. FebriDx was found to be useful, especially for predicting the etiology of pneumonia in children [107], which is influenced by different biomarkers [108]. Importantly, a recent ease-of-use assessment of the ability of FebriDx to guide antibiotics for lower respiratory tract infections showed good user-friendliness [92]. Furthermore, patients and healthcare providers express their positive views on the test's usefulness in antibiotic prescription decisions for lower respiratory tract infections [109]. An economic evaluation of the use of FebriDx to guide antibiotics for acute respiratory tract infection estimated a 27% reduction in the national cost in the United Kingdom (UK) [10] and a 30% reduction in the cost in the United States of America (USA) [110]. This individual dual marker test costs approximately £12.75, similar to CRP POCT cartridges [92].

Considering the limitations of using MxA alone for antibiotic guidance is essential since no randomized controlled trials are available. However, when combined with CRP (in FebriDx), it helps in antibiotic prescription guidelines thus reducing inappropriate treatments [111].. Although further studies on its clinical importance are needed, FebriDx MxA combined with other bacterial biomarkers can be used as an integral component in antibiotic stewardship efforts in which rapid and accurate diagnostic tools to differentiate between bacterial and viral infections are urgently needed.

The lack of clear regulatory body advice for tests distinguishing between bacterial and nonbacterial infections based on host biomarkers is another crucial problem, especially in resource-limited nations. Importantly, the presence of confounding factors, such as HIV, malaria, helminths, and malnutrition might affect the performance of MxA, and real-world evaluation would be interesting.

Other promising host-derived biomarkers with similar roles are also highlighted in Table 2. However, sufficient data representing diagnostic performance and clinical value under different conditions are lacking. In addition, these markers are not translated into a point-of-care format.

Conclusion and future perspectives

Given that the use of host-derived biomarkers for differentiating viral from bacterial infection is challenging, the use of MxA could be vital for diagnosing acute viral infection, thereby reducing the overuse of antibiotics. However, studies based on real-world evaluations of MxA are needed and would be of great interest to the scientific community. Confounding factors such as HIV, malaria, helminthic infections and malnutrition might affect the performance of MxA and hence performance evaluation under these conditions would be helpful to understand its usefulness in resource-limited settings. Since we are in the era of antimicrobial resistance, extensive research on the development and validation of other alternative biomarkers that robustly identify and differentiate viral from bacterial infection is urgently needed. Moreover, further research is needed to fully understand the role of MxA in autoimmune disease, cancer, and pulmonary fibrosis. In addition, the inhibition of MxA production by viruses such as dengue virus could be a promising future target strategy for the design of antiviral therapies. In conclusion, elevated MxA combined with several other biomarkers, such as CRP and PCT, is a promising tool for identifying patients with viral infections. However, extensive ongoing real-world evaluation and well-designed randomized controlled studies are desirable to assess the reliability and clinical utility of this test in clinical settings.

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