

Outpatient Antibiotic Stewardship: A Growing Frontier—Combining Myxovirus Resistance Protein A With Other Biomarkers to Improve Antibiotic Use

Patrick Joseph¹ and Eliot Godofsky²

¹Private Practice to California Infection Control Consultants, San Ramon, California; ²University Hepatitis Center, Bradenton, Florida

Background. The majority of oral antibiotics are prescribed in outpatient primary and urgent care clinics for acute respiratory infections. Effective antibiotic stewardship must include proper prescribing for outpatients as well as for those in a hospital or long-term care facility.

Methods. Major databases, including MEDLINE and the Cochrane Library, were searched for prospective human clinical studies, including children and/or adults published between January 1966 and November 2017 that evaluated Myxovirus resistance protein A (MxA) as a biomarker for diagnosing viral infections as well as both C-reactive protein (CRP) and procalcitonin (PCT) as potential biomarkers for identifying and differentiating true bacterial upper respiratory infection (URI) from colonization.

Results. Ten prospective human studies, totaling 1683 patients, were identified that evaluated MxA as a viral biomarker in children and/or adults. Both systematic review articles, meta-analyses, and randomized controlled clinical trials that examined CRP and/or PCT as a biomarker for identifying clinically significant bacterial infections and supporting antibiotic stewardship were identified.

Conclusions. Quick and accurate differentiation between a viral and bacterial respiratory infection is critical to effectively combat antibiotic misuse. MxA expression in peripheral blood is a highly specific marker for viral infection. Combining MxA with other inflammatory biomarkers to test for respiratory infections offers enhanced sensitivity and specificity, forming an excellent tool for antibiotic stewardship in the outpatient setting.

Keywords. C-reactive protein (CRP); diagnostic; Febrile; myxovirus resistance protein A (MxA); point of care (POC); procalcitonin (PCT); upper respiratory infection (URI).

Acute upper respiratory infections (URIs), inclusive of acute pharyngitis, sinusitis, and tracheobronchitis, are the most common reason for antibiotic therapy in primary care and urgent care settings and account for nearly 90% of the 41 million antibiotic prescriptions per year for acute respiratory infections (ARI) in the United States [1]. Unfortunately, viral respiratory infections like influenza often lead to inappropriate prescribing of antibiotics, contributing to the expanding problem of microbial resistance. In addition, routine antibiotic overuse can lead to unnecessary consultations, emergency room (ER) visits, allergic reactions including anaphylaxis, Stevens Johnson syndrome, and *Clostridium difficile* infection [1].

Clinical differentiation between a viral and bacterial URI can be challenging. Diagnostic uncertainty, combined with patient

or family pressures, frequently facilitates the misuse of antibiotics [1]. Acute pharyngitis is primarily viral in adults, with only about 10% of patients having a bacterial cause, most commonly a group A beta-hemolytic streptococcus (GABHS) [2]. None of the symptoms, physical findings, or the clinical criteria scores are highly specific for differentiating GABHS from non-GABHS causes [3]. Despite the relatively high frequency of viral pharyngitis in adults, physicians prescribe antibiotics for 78% to 98% of patients with clinical pharyngitis in an effort not to miss bacterial GABHS pharyngitis [4, 5]. Furthermore, even though 90% of acute bronchitis is thought to be of viral etiology in the United States, the rate of antibiotic prescribing was shown to be between 60% and 80% [6].

Rapid antigen testing, cell culture, and newer molecular tests are limited by their cost, availability, and inability to differentiate microbial colonization or carrier states from clinical infection [7]. This may result in unnecessary antibiotic prescriptions, underscoring the importance of accurately defining a clinically significant bacterial infection [3, 8, 9].

Confirmation of a clinically significant active infection requires the identification of an infectious agent via antigen detection, culture growth, or molecular techniques in association with a positive immune response, whereas the lack of a systemic immune response suggests a carrier state or colonization.

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Correspondence: P. Joseph, MD, FIDSA, 5601 Norris Canyon Road, Suite 220, San Ramon, CA 94583 (pjoseph@cicc.net).

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Traditionally, paired serology is performed, necessitating 2 patient visits 2–4 weeks apart, and thus is impractical. Antigen testing and molecular tests are more time efficient [10] but may overestimate the prevalence of true infection, leading to the prescription of unnecessary antibiotics [7, 8].

Efficiently defining a clinically significant bacterial infection requiring antibiotic therapy is the rate-limiting step of antibiotic stewardship in the outpatient setting. Biomarkers such as C-reactive protein (CRP) or procalcitonin (PCT) independently may identify clinically significant infections, thereby reducing the risk of missing a clinically significant bacterial infection. However, these biomarkers lack adequate specificity to differentiate a viral from a bacterial infection and ultimately lead to antibiotic overtreatment of viral infections. Myxovirus resistance protein A (MxA), a protein induced by type I interferon, is selectively elevated in patients with viral infections and has the potential to greatly enhance the rapid distinction between viral and bacterial respiratory infections [7, 8]. Combining CRP or PCT with an elevated MxA will help identify patients who most likely have viral infection, allowing physicians to consider reserving antibiotics in this patient population and proceed with a watchful, waiting strategy.

METHODS

Major databases, including MEDLINE and the Cochrane Library, were searched for prospective human clinical studies, including children and/or adults published between January 1966 and November 2017 that evaluated Myxovirus resistance protein A (MxA) as a biomarker for diagnosing viral infections as well as both C-reactive protein (CRP) and procalcitonin (PCT) as potential biomarkers for identifying and differentiating true bacterial upper respiratory infection (URI) from colonization.

Colonization and the Carrier State

Both viruses and bacteria may colonize the nasopharynx (NP) and oropharynx (OP) without causing infection. Advances in molecular testing and microbial antigen detection with enhanced sensitivity may allow detection of colonization or postinfectious shedding of respiratory pathogens without clinical significance [10]. Respiratory viruses, such as the herpes viruses, including Epstein-Barr (EBV) [11], herpes simplex virus (HSV) [12], and cytomegalovirus (CMV) [13], are associated with chronic intermittent asymptomatic nucleic acid shedding.

Streptococcal carriers are at low risk to spread GABHS to close contacts. They do not require antibiotic treatment and are at minimal risk for development of rheumatic fever [14]. Streptococcal carriage may persist for many months and frequently poses diagnostic challenges when a symptomatic viral URI develops in carriers. The low predictive value of throat swabs relates to the prevalence of carrier rates [15], and neither

the blood agar plate culture nor the rapid antigen tests can accurately differentiate individuals with true GABHS pharyngitis from GABHS carriers [16]. Studies have shown that only 40%–50% of the children with GABHS isolated from the upper respiratory tract who presented with symptoms of tonsillitis or pharyngitis demonstrated a systemic immune response [16–18].

When Group A strep is cultured from the OP and associated with an antibody response characteristic of a true infection, CRP will elevate 80%–90% of the time [15, 17]. Conversely, patients with a negative initial CRP test seldom show a rise in antibody titer [19], and 96% have CRP <10 mg/mL [20]. The high carrier rate of GABHS and false-positive diagnoses may contribute to the apparent “failure” rate of approximately 20% with penicillin therapy [21]. Valkenburg et al. have shown that an antistreptococcal antibody titer is more accurate than a throat culture in predicting therapeutic outcome [22].

Differentiation of infection from colonization requires the demonstration of an antibody response. However, proving this immune response is time-consuming and may lead to false-negative results following appropriate antibiotic therapy [23]. A study by Ivaska et al. [3] showed that in 83 patients presenting with pharyngitis, there was no significant difference in the mean initial serum antistreptolysin O (ASO) levels between the GABHS and non-GABHS patients and only 5 patients showed a 2-fold ASO increase in paired serum samples. Of the 5 patients with an antibody response, 3 of them were GABHS positive, 1 of them was GCBHS positive, and 1 was negative for streptococci by throat culture. Conversely, blood MxA levels were found to be elevated in 79% of patients with viral pharyngitis and remained low in 90% of patients with GABHS without virus detection [3].

Serological Biomarkers

Myxovirus Resistance Protein A

MxA expression in peripheral blood is a highly specific marker for viral infection [24–30]. MxA is an intracellular blood protein that mediates cellular resistance against a wide range of viruses and elevates in the presence of most acute active viral infections including influenza A and B, respiratory syncytial virus, parainfluenzaviruses, Epstein-Barr, herpes simplex, cytomegalovirus, adenovirus, coronavirus, rhinovirus, and metapneumovirus infections. However, it is not specific to a particular type of virus [7, 25–27, 30].

Interferons (IFNs) are naturally occurring proteins that are an important part of the host’s innate defense mechanisms and are released in response to viral infections [7, 31]. The MxA gene is expressed in blood mononuclear cells or locally in tissues, and expression is upregulated exclusively by type I IFNs [25, 26]. The MxA gene does not respond to other cytokines such as IL-1 or TNF- α . Neither type I IFN nor MxA elevates in healthy patients or those presenting with bacterial infections [7].

In most cases of acute viral infections, type I IFN and MxA are released into the peripheral blood. Detection of interferons in serum is difficult and unreliable, mainly due to their short half-life [30]. In contrast, MxA has a long half-life of 2.3 days, low baseline level of less than 15 ng/mL, and a fast induction time of 1–2 hours after infection [32]. The low basal levels of MxA protein in tissues, its exclusive expression by type I IFNs, and its relatively long half-life make it an excellent biomarker for systemic IFN- α/β production in viral infections.

Recent studies show that other viral biomarkers, such as TNF-related apoptosis-inducing ligand (TRAIL) and IP-10, are less effective than MxA at differentiating viral infection. TRAIL and IP-10 show area under the curve (AUC) specifically for viral infection of 0.72 and IP-10 of 0.72 [33]. Other studies further support the superiority of MxA as a biomarker for identifying a viral infection, especially in URI [3, 24].

C-Reactive Protein

CRP is a nonspecific, acute-phase protein that increases during an inflammatory process such as a severe infection. The normal CRP serum concentration is less than 1–3 mg/L and can rise above 500 mg/L in the presence of severe inflammation or infection [7]. A high CRP level generally indicates bacterial rather than viral infection and can also be used to assess disease severity. A systematic review of acute rhinosinusitis showed that a CRP of less than 10 mg/L provided evidence against bacterial sinusitis and a CRP greater than 20 mg/L showed evidence supporting bacterial sinusitis [34]. Calvino et al. showed that CRP elevated above 20 mg/L in nearly all cases of GABHS, ensuring that a clinically significant infection would less likely be missed, but could not differentiate viral from bacterial infection [35]. Similarly, Putto et al. found that in examining 62 children with positive bacterial cultures, 89% showed a CRP elevated over 20 mg/L, consistent with a clinically significant bacterial infection [36].

Typically, bacterial infection stimulates/elevates CRP while having no impact on MxA levels [29]. CRP elevates within 4–6 hours of infection, doubles every 8 hours, and peaks at approximately 36–50 hours [37]. Although less common than bacterial infection, viral pathogens such as adenovirus, parainfluenzavirus, influenza, respiratory syncytial virus, Epstein-Barr virus, herpes simplex virus, and varicella zoster virus can raise CRP levels significantly over 20 mg/L [7, 24–27, 30, 38]. Therefore, a test system that utilizes both CRP and MxA simultaneously can potentially differentiate viral infections from bacterial disease as the elevated CRP from a viral infection would also be associated with an elevation in MxA, whereas the MxA levels would be normal in bacterial infection.

Procalcitonin

PCT is the peptide precursor of calcitonin, a hormone that is synthesized by the parafollicular C cells of the thyroid and regulates calcium homeostasis. Standard reference values of PCT in

adults and children older than 72 hours are usually 0.15 ng/mL or less [39]. In response to inflammation associated with bacterial endotoxin or inflammatory cytokines, PCT elevates within 2–6 hours, peaks at 12–24 hours, and has a half-life of 25–40 hours [37, 40]. Higher procalcitonin levels in patients with bacterial sepsis are associated with a greater likelihood of severe sepsis, septic shock, and decreased survival [40]. Colonization or carrier states without a systemic host response do not significantly raise procalcitonin levels [41]. Procalcitonin levels fall with successful treatment of either severe bacterial infection or noninfectious inflammatory stimuli [40].

URIs tend to cause modest elevations in PCT [42, 43]. Using a lower PCT threshold of 0.1 ng/mL in association with polymerase chain reaction–confirmed bacterial cultures of common oral pathogens such as GABHS or atypical pathogens such as *Chlamydia* or *Mycoplasma*, would suggest a true active bacterial infection. Higher PCT cutoffs of 0.15–0.25 ng/mL could be used in association with growth of typical bacterial colonizers or in association with a negative bacterial culture to suggest active bacterial infection in patients without another confirmed source of infection, such as a viral infection [8, 40]. The PCT response to viral infections and noninfectious inflammatory stimuli such as autoimmune disease and chronic inflammatory processes typically do not exceed 0.75 ng/mL [44, 45]. Branch et al. found that 17% of viral infections had a PCT >0.25 ng/mL [46]. At low concentrations (<1.0 ng/mL), PCT is inadequate by itself to differentiate viral from bacterial etiology [8, 47, 48].

RESULTS

Ten prospective human studies, totaling 1683 patients, were identified that evaluated MxA as a viral biomarker in children and/or adults. Both systematic review articles, meta-analyses, and randomized controlled clinical trials that examined CRP and/or PCT as a biomarker for identifying clinically significant bacterial infections and supporting antibiotic stewardship were identified.

Clinical Outcomes Using Biomarker Guidance

CRP and PCT levels do not correlate consistently with each other, but in primary care patients with URI, each has moderate predictive value for clinical outcome [49]. Both CRP and PCT have been shown to elevate in infectious pharyngitis; however [42, 43], CRP is more sensitive and PCT is more specific for detection of bacterial tonsillopharyngitis [43]. In most acute respiratory infections, including URI, antibiotic therapy based on either biomarker alone has led to reduced antibiotic prescriptions without increased morbidity [50–54].

Numerous studies have delineated the utility of CRP in antibiotic stewardship, specifically decreasing antibiotic prescriptions for patients with respiratory tract infections [50–53]. In Europe, a CRP >20 mg/L is recommended in the Pneumonia Guidelines by the National Institute for Health and Care Excellence (NICE) as a trigger for prescribing antibiotics [50].

Using this CRP threshold, there was no statistically significant increase in patient consultations, emergency visits, or adverse outcomes [50]. In a recent systematic review and meta-analysis of 13 studies in primary care including 10 005 patients, CRP testing led to significantly reduced antibiotic prescribing at the index consultation without increasing morbidity [51].

The effect of CRP testing on the outcome of patients in general practice was evaluated in a recent randomized clinical trial. A total of 179 patients were included, 101 in the CRP measurement group and 78 in the control group. Results suggested that CRP testing in patients with acute cough may reduce antibiotic prescribing and referral for radiography without compromising outcome [55]. Similar results have been demonstrated in identifying patients with chronic obstructive pulmonary disease (COPD) exacerbations who do not need antibiotic treatment [56].

Clinical trials using PCT to guide antibiotic therapy for patients with acute respiratory tract infections have shown that a biomarker-driven algorithm can decrease antibiotic prescribing significantly and without an increase in adverse events or treatment failures [57–62]. PCT-guided antibiotic stewardship reduced initial antibiotic prescription rates by 40% to 50% in patients with lower respiratory infection (LRI) presenting to the emergency departments [61], 70% to 80% in ambulatory patients presenting to their general physicians [63], and reduced total antibiotic exposure in community-acquired pneumonia by 40% to 50% [64]. In a single-center randomized controlled study, a significant reduction in antibiotic use in patients hospitalized with severe acute exacerbations of asthma was shown utilizing an algorithm of PCT measurements. In this study, withholding antibiotic treatment did not cause any apparent harm [65].

The Role for Combining Biomarkers to Guide Outpatient Antibiotic Prescribing

Several clinical studies have verified that high MxA protein levels are strongly correlated with a systemic viral infection while elevated CRP levels are more closely associated with bacterial disease [3, 7, 8, 24–26]. Simultaneously performing CRP and MxA should predictably increase sensitivity and specificity for identifying bacterial disease.

Combining MxA detection with a marker specific to bacterial infection, such as CRP, could be of greater predictive value and allow more reliable differentiation between viral and bacterial infections than using a marker of bacterial infection alone [7, 8, 24]. A high MxA with or without an elevated CRP would strongly suggest a viral infectious process and the absence of a bacterial infection [24]. Unlike the common occurrence of incidental identification of multiple pathogens in the OP or NP, true active co-infection that leads to a systemic viral and bacterial immune response is not common in URI [1, 8]. If both viral and bacterial pathogens are identified, an associated PCT ≥ 0.75 ng/mL or CRP ≥ 100 mg/L may support a diagnosis of a true co-infection result. Others have suggested that a defined

ratio of CRP/MxA would optimize differentiation between a viral and bacterial infection [3].

Rapid CRP tests are shown to promote more prudent use of antibiotics in primary care and have led to a 19% reduction in antibiotic prescriptions [66]. A prospective, multicenter, cross-sectional study of adults and children with febrile URIs evaluated the diagnostic accuracy of a 15-minute, single-use disposable immunoassay that includes both CRP and MxA (FebriDx; RPS Diagnostics, Sarasota, FL) [8]. During a multicenter, US-based study that enrolled 370 patients, 205 symptomatic patients with URI and 165 asymptomatic patients from 10 clinical sites, including academic emergency departments and community care centers, demonstrated a 97% negative predictive value (NPV) for bacterial infection. Also, the use of CRP independent of MxA would have led to overtreatment of 38% of viral infections [8]. The pattern of results from test systems with CRP or PCT combined with MxA may assist health care professionals to identify an immune response to a suspected viral and/or bacterial infection and greatly enhance antibiotic stewardship in the outpatient setting [7, 24]. This was recently demonstrated in a FebriDx study of 21 children and adults (mean age = 46 years) that evaluated the use of MxA plus CRP as a guide for outpatient antibiotic management. Therapy was altered in 48%, and unnecessary antibiotic prescriptions were reduced by 80% without any adverse effects [9].

CONCLUSIONS

The value of inpatient antibiotic stewardship is embraced by many professional societies such as the IDSA and the Center for Disease Control and Prevention (CDC). There are presently several recommendations, guidelines, and requirements from some licensing organizations that continue to be updated as diagnostics evolve and outcome measurements improve [67]. The current focus on inpatients fits well with existing hospital surveillance programs and the ability to identify multiply drug-resistant organisms.

Inpatient stewardship is driven in part by modern microbiology, which is often not available or impractical in the outpatient setting. Outpatient stewardship is also critical, but more difficult to implement. According to the CDC and American College of Physicians, up to 50% of antibiotic courses prescribed in the outpatient setting are inappropriate and completely unnecessary. This equates to more than \$3 billion in direct costs. Additionally, billions of indirect costs include: (1) antibiotic-resistant illnesses, (2) antibiotic adverse events, and (3) secondary infections with *Clostridium difficile* diarrhea [1].

A major obstacle to effective outpatient antibiotic stewardship and appropriate antibiotic prescribing is the difficulty in accurately defining the microbial cause of respiratory infections, especially distinguishing viral from bacterial etiology. This diagnosis is even more challenging when rapid tests identify bacterial pathogens present in the carrier state. Additionally, attempts to

differentiate bacterial from viral infections using only the history and physical findings are inaccurate about one-half of the time [64]. Patients may also have symptoms mimicking an infectious process, which is actually caused by hypersensitivity, especially rhinovirus and coronavirus acting directly as allergens, eliciting an IgE elevated response. This may lead to low-grade fevers and exacerbation of reactive airway disease in predisposed patients with a history of underlying allergies, atopy, asthma, or COPD [68, 69]. Lastly, patient expectations of therapy present another challenge to overcome in the outpatient setting. In 1 study, up to 50% of parents had a previsit expectation of receiving an antibiotic [70]. When patients expect or demand antibiotics, they are more likely to receive them even though patients' satisfaction may not be affected by prescribing of antibiotics [53].

One approach to improve appropriate outpatient antibiotic use is to supplement the history and physical examination with point-of-care tests for selected biomarkers. Biomarkers such as CRP, PCT, and MxA respond differently to the host immune response and can help distinguish viral from a bacterial infection, including noninfectious causes of symptoms. At low levels, CRP and PCT are sensitive but not specific to bacterial infection, while at high levels, both CRP and PCT become more specific to bacterial infection. Although PCT and CRP are not specific enough to differentiate a viral from bacterial infection, these biomarkers in combination with MxA substantially improve the differential diagnostic accuracy [7, 9, 24]. The combined interpretation of MxA with either CRP or PCT dramatically improves both sensitivity and specificity for differentiating a viral from bacterial infection [7, 8].

A rapid point-of-care test that measures both CRP and MxA is available in Europe and Canada, but not currently in the United States. Utilization results of the FebriDx test in outpatient clinical practice are impressively encouraging, including diagnostic accuracy and positive impact on appropriate antibiotic prescribing [9]. The 97% NPV reduces the clinician's fear of missing a serious bacterial infection and supports watchful waiting, while the ability to demonstrate tangible results at the office visit can relieve patient pressures for antibiotic prescriptions [9]. A recent survey estimates that 85% of US primary care clinicians currently use a rapid strep test and 60% use a rapid flu test, oftentimes in the same patient [71]. A rapid point-of-care test utilizing detection of both MxA and CRP without any required ancillary reader equipment would likely reduce the need for rapid strep and flu testing and provide direct cost savings while reducing indirect costs related to the cost of unnecessary antibiotics themselves, adverse events, and potential resistance.

In summary, it is critically important that antibiotic stewardship rapidly move into the outpatient setting. Diagnostic algorithms based solely on history and physical are historically inaccurate for many common outpatient syndromes. Incorporating point-of-care testing with a combination of

biomarkers such as CRP and MxA can be extremely useful as an important adjunct to traditional methods. Further studies should be directed toward the delineation of biomarker utility in the outpatient setting.

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