

REVIEW

Serum biomarkers in elderly asthmaJoão Rufo, MSc¹, Luís Taborda-Barata, MD, PhD^{1,2}, and Olga Lourenço, PhD¹¹CICS-UBI, Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal and ²Department of Allergy and Clinical Immunology, Cova da Beira University Hospital, Covilhã, Portugal**Abstract**

Objective: Asthma is usually misdiagnosed and under-treated in the elderly population, resulting in complications and increased severity to the patient. In this review, we describe some of the most important serum markers of asthma studied so far, reporting their outcomes and possible prediction of asthma in the elderly population. **Methods:** The PubMed electronic database was used to search for promising serum biomarkers of asthma studied in original articles published in peer-reviewed journals from 2000 to January 2013. **Results:** A total of 13 relevant serum biomarkers were selected, including IgE, CRP, high sensitive CRP, IL-6, IL-8, IL-17, TNF- α , neopterin, serum amyloid A, eosinophil cationic protein, leukolysin, YKL-40 and soluble CD86. **Conclusions:** Although the major focus of treatment and research has been on allergic asthma, several forms of the disease are recognized, such as neutrophilic asthma, which is characteristic of older patients. Different phenotypes imply different treatments and so it becomes important to correctly determine which type of asthma the patient is suffering from. Serum markers capable of supporting a diagnosis of asthma are needed in order to counter mistreatment and misdiagnosis with other obstructive airways disease (OAD) in elderly patients. As convenient as serum markers may seem to be, a marker capable of accurately identifying asthma with sufficient specificity is yet to be found.

Keywords

Diagnostic, geriatric, mechanisms, phenotypes, severity

History

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Introduction

Asthma can affect all ages and it is often misdiagnosed, under diagnosed and under-treated in elderly patients [1]. There is an estimated proportion of 6.5–17% sufferers of asthma in this age group, and deaths resulting from it occur mostly among these patients [2]. This mortality rate is often associated with poor diagnosis and inadequate treatment. The complexity of the disease, with variable clusters of phenotypes and endotypes along with symptoms similar to those of other obstructive airways disease (OAD) and possible comorbidities are the principal obstacles to the diagnosis of asthma [1–4]. In addition, lung capacity of individuals over 65 years of age tends to decrease by about 40% over time [5]. This loss of lung function may compromise diagnostic tests, as it produces a characteristic change in the flow-volume curve which is typical of small airways disease [4]. In addition, different phenotypes of asthma may be more difficult-to-treat, more severe and unresponsive to corticosteroids, showing more extensive structural changes [6].

Although several distinct forms of asthma are recognized, the major focus of treatment and research has been on allergic asthma [7]. This phenotype of asthma is characterized by the presence of allergen-specific Th2 cells in the lungs of

asthmatic patients [8], producing cytokines such as IL-4, IL-5, IL-9 and IL-13. Another characteristic of allergic asthma is the abnormally high levels of serum allergen-specific IgE possibly due to the action of augmented IL-4 production, as well as a significant enhancement in eosinophilia in serum and bronchial mucosa, most likely due to the effects of increased IL-5 production [9].

Non-allergic asthma is a less common phenotype as it accounts for two of every five cases of asthma in adults and constitutes both in terms of symptoms and lung function, a more severe form of the disease [10]. This form of asthma tends to be more frequently associated with higher numbers of serum and sputum neutrophils and is characteristic of older patients with a late onset of the disease. Additionally, it shows reduced response to corticosteroids and has a more severe and abrupt onset rather than progressing from mild to moderate form [6,11]. This more neutrophilic cellular phenotype of asthma is characterized by a less apparent Th2-type response when compared to other phenotypes, and tends to be more frequently associated with an increased Th1-type inflammatory response. This increment in a Th1-type response may be correlated with the increased neutrophilia, since IL8-mediated neutrophil influx is typically associated with activation of the innate immune system, thereby suggesting that neutrophilic asthma may be involved in its activation [12–14].

Elderly patients with non-allergic asthma may have low serum IgE levels as a consequence of immunosenescence,

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thus making it even harder to diagnose [15], and their symptoms are sometimes mistaken for those of chronic obstructive pulmonary disease (COPD) [16]. In fact, both asthma and COPD are major chronic OAD that involve underlying airway inflammation and which may be difficult to differentiate, particularly in the elderly. Unlike asthma, airflow limitation in COPD is not fully reversible and is usually progressive, but these symptoms may be hard to distinguish from those of asthma, especially in elderly patients and smoking asthmatics [17].

Currently, the clinical diagnosis of asthma is based on the medical history and recurrent symptoms of the patient, on measurements of lung function (such as spirometry), and on non-invasive markers of airway inflammation (FeNO and FeCO). Biomarkers for asthma can be found in serum, sputum, bronchial alveolar lavage (BAL) and bronchial biopsies. Bronchial biopsies and BAL have the highest diagnostic value for asthma. However, they require extremely invasive and complicated techniques, thus becoming hard to use as a routine diagnostic tool. The fraction of exhaled Nitric Oxide (FeNO), on the other hand, is a less invasive assay that measures the exhaled nitric oxide, thereby indirectly assessing the level of inflammation in the airways. Nevertheless, the level of diagnosis based upon FeNO decreases in patients with smoking habits or other OAD [18,19]. Serum biomarkers are easily assessable and may be easily quantified, needing only blood samples to be retrieved from the patients. Due to the strong association between asthma and allergic rhinitis, the clinical practitioner often requests total and specific IgE measurements, which is currently the only serum marker used with some frequency for the clinical diagnosis of asthma [20]. However, the existence of a profile including several serum biomarkers specifically related to asthma would greatly improve the diagnostic efficiency and monitorization of the disease. Furthermore, due to their convenience, serum biomarkers could become an important option for clinical practice.

In the next section, we describe several clinical research studies based on various serum markers and their relevance to the diagnosis and/or monitorization of asthma in elderly patients.

Methods

The PubMed electronic database was used to identify serum biomarkers of asthma measured in previously published studies, using the search terms “serum markers asthma,” “elderly asthma,” and “neutrophilic asthma.” The search was limited to studies published between 2000 and January 2013. We focused on articles including elderly populations and selected those showing better results. However, since there were very few studies regarding serum markers of elderly asthma, we also included some studies including young adult populations. We selected only peer-reviewed journal articles, excluding conference abstracts, reports, and books.

Results

A total of 13 relevant markers were selected after analyzing the results of 18 original studies. These studies are summarized in Table 1, sorted by studied biomarkers.

IgE

Atopic patients over express IgE, namely to common allergens, which makes them more prone to developing allergic diseases. In this regard, IgE may be a crucial factor underlying airway hyper-responsiveness in asthma. Measurement of total serum IgE was one of the first tests used to study allergic inflammation and it was thought to be a good and easily measured marker of asthma, since asthmatic patients tend to be allergic [21]. However, this was proven to be not necessarily true. It may help to exclude a diagnosis of COPD since the inflammatory response in this disease is associated with a lower concentration of serum IgE when compared with asthma [22], but despite the good sensitivity, total serum IgE has a poor specificity and negative predictive value as other diseases may also have increased IgE levels. This is the case of patients with helminthiasis or other allergic diseases, such as allergic dermatitis [23–26]. In addition, in elderly patients, total serum IgE levels are generally lower than in younger adults, which may decrease the sensibility of this test. Overall, determination of total serum IgE levels in elderly asthmatic patients has to be interpreted with caution.

In contrast, the quantification of allergen-specific IgE (sIgE) may be clinically relevant to the diagnosis of allergic diseases. It measures the circulating levels of IgE in response to a specific allergen. This can be quantified through an IgE auto-analyzer, such as Phadia® (ImmunoCAP), which measures the response of sIgE to specific allergens, thus determining the atopic status of a patient. In addition, sIgE may be indirectly measured in the skin using *skin-prick-tests* (SPT). SPT measures mast cells degranulation on the skin in response to certain allergens, thus obliquely quantifying the sIgE for that allergen. The SPT method has a great diagnostic value, but the lack of full standardization, plus adverse skin topography in certain patients, reduces its value in clinical research [27,28]. An asthmatic patient can be regarded as atopic when there is positive hyper-responsiveness to at least one allergen, in a SPT, or sIgE test results are greater than 0.35 PAU/l. King et al. (2004) performed a study in elderly asthmatics (33 asthmatics, 21 healthy controls), and showed that levels of allergen-specific IgE (sIgE) to cat, ragweed, German cockroach, *Dermatophagoides pteronyssinus* and oak allergens, only sIgE for *Dermatophagoides pteronyssinus* was significantly higher in asthmatic than in non-asthmatic patients [29].

Due to the different aspects of SPT and sIgE quantification, both tests are recommended as diagnostic tools for detecting asthma and atopy.

CRP and hs-CRP determination

Acute phase proteins (APP) tend to be elevated in the serum of patients suffering from infections or other inflammatory conditions [30]. C-reactive protein (CRP), which is one of the most studied and easily measurable positive APP, is known to be over expressed in the presence of systemic inflammation. Since asthma is regarded as an inflammatory disease of the airways, Buyukozturk et al. (2004) tested CRP as a potential marker of asthmatic inflammation, in a population of 50 adult patients with rhinitis, 20 adult patients with asthma and 20

Table 1. Summary of the analyzed studies, sorted by biomarkers.

Markers	Study	Population (mean age)	Outcome
IgE (total)	[22] ^a	86 patients with asthma (38.0); 36 with COPD (56.8); 13 with partial reversibility (57.9); 75 HC (42.3).	Large overlapping of serum total IgE was observed in both allergic and HC populations. Good sensibility, poor specificity and poor negative predictive value.
	[29] ^a	33 elderly asthmatics (65–90); 21 elderly HC (65–90).	
	[23] ^b	771 asthmatics (7.8 and 30.1); 117 HC (6.6 and 30.7).	
IgE (specific)	[29] ^a	33 elderly asthmatics (65–90); 21 elderly HC (65–90).	IgE for DerP was higher in asthmatic than in non-asthmatic patients.
CRP	[16] ^c	96 patients with asthma exacerbations (18–84); 62 with pneumonia (24–93); 161 with COPD exacerbations (39–93).	No significant difference between CRP levels in COPD, asthma exacerbations and HC.
	[31] ^a	50 patients with rhinitis (32); 20 asthmatics (36); 20 HC (31).	
Hs-CRP	[32] ^a	26 asthmatics treated with ICS (33.3); 24 ICS naive (35.1); 15 HC (matching age).	Hs-CRP was significantly increased in asthmatic patients, compared to HC, even under ICS treatment.
	[33] ^a	106 NNA (57.4); 26 NA (62.6); 83 HC (61.3)	Hs-CRP levels were not elevated in NNA. Hs-CRP increased in NA.
	[22] ^a	86 patients with asthma (38.0); 36 with COPD (56.8); 13 with partial reversibility (57.9); 75 HC (42.3).	Hs-CRP was significantly increased in patients with COPD, compared to asthmatics and HC.
IL-6	[33] ^a	106 NNA (57.4); 26 NA (62.6); 83 HC (61.3)	IL-6 levels were significantly increased in both asthmatic groups when compared to HC, and were significantly increased in NA when compared to NNA.
IL-8	[33] ^a	106 NNA (57.4); 26 NA (62.6); 83 HC (61.3)	IL-8 was significantly increased in NA when compared to NNA and HC.
	[48] ^a	22 mild/moderate asthmatics (44.5); 14 severe asthmatics (56.6); 20 HC (43.4).	IL-8 levels were significantly higher in both asthmatic groups, and in severe asthmatics IL-8 levels were significantly higher than in mild/moderate asthmatics. Severe asthmatics were significantly older.
	[49] ^a	13 asthmatics (54.3); 25 with COPD (64.0)	No significant differences were found between serum IL-8 levels of asthma and COPD.
IL-17	[58] ^a	23 patients with mild asthma (41.4); 26 with moderate asthma (46.6); 36 with severe asthma (50.8).	Patients with severe asthma were significantly older than patients with mild asthma. IL-17 was significantly increased in severe asthma compared to mild/moderate forms.
TNF- α	[33] ^a	106 NNA (57.4); 26 NA (62.6); 83 HC (61.3)	TNF- α showed no significant differences between NA, NNA and HC.
	[48] ^a	22 mild/moderate asthmatics (44.5); 14 severe asthmatics (56.6); 20 HC (43.4).	TNF- α was significantly increased in severe asthmatics compared to other asthmatics and HC.
Neopterin	[60] ^a	42 allergic asthmatics and 22 non-allergic asthmatics (72.4); 41 HC (79.2).	Neopterin was increased in non-allergic asthmatics in comparison with allergic asthmatics and HC.
SAA	[31] ^a	50 patients with rhinitis (32); 20 asthmatics (36); 20 HC (31).	Patients with asthma showed significantly higher SSA levels compared to HC.
	[37] ^c	17 asthmatics BT (35.5); 10 asthmatics AT (30.0); 10 HC (31.0).	
ECP	[36] ^a	441 patients with RD (57.0); 33 HC (63.0).	ECP was significantly higher in patients with RD compared to healthy volunteers. No correlation was found between serum ECP value and PBE absolute number. The predictive model was significant only for asthma, with a sensitivity of 70% and a specificity of 74% (efficiency 73%).
	[29] ^a	33 elderly asthmatics (65–90); 21 elderly HC (65–90).	ECP levels were not significantly different between elderly asthmatic and HC.
	[37] ^c	17 asthmatics BT (35.5); 10 asthmatics AT (30.0); 10 HC (31.0).	ECP was significantly increased in asthmatics BT compared to HC and asthmatics AT.
Leukolysin	[73] ^a	337 patients from nuclear families with asthma (28.1); 122 “married-in” individuals (48.8).	Changes in serum leukolysin were closely paralleled to those of total IgE in both atopic asthmatics and COPD.
YKL-40	[66] ^a	59 asthmatics (23–76); 29 HC (20–80).	YKL-40 was significantly increased in asthmatics compared to HC. No relations were found between YKL-40 level and asthma severity, control, or total serum IgE.
	[64] ^a	Three cohorts with a total of 194 patients with asthma (44.0) and a total of 59 HC (42.0)	YKL-40 was significantly increased in asthmatics compared to HC. Serum YKL-40 correlated positively with total serum IgE and PBE.
	[65] ^d	16 patients with stable asthma (30.9); 46 with asthma exacerbations (43.3); 64 HC (42.2).	
	[67] ^c	40 asthmatics (47.0); 13 non-smokers (50.0); 16 smokers (55.0).	Serum YKL-40 was significantly increased in asthmatics compared to controls, and correlated with bronchial smooth muscle cells migration.
sCD86	[43] ^a	24 patients with stable asthma (39.2); 28 with aggravated asthma (42.2); 25 HC (38.1).	sCD86 was significantly increased in acute asthmatics compared to stable and HC. No significant differences were found between CD86 levels in stable asthmatics and HC.

Type of study: ^aCross-sectional; ^bRetrospective; ^cProspective; ^dCase-control.

HC, healthy controls; BT, before treatment; AT, after treatment; NA, neutrophilic asthmatics; NNA, non-neutrophilic asthmatics; PBE, peripheral blood eosinophils; ICS, immunocorticosteroids; RD, respiratory diseases.

healthy controls with matching age. These authors concluded that the mean CRP levels of patients with asthma and rhinitis were not significantly different from those of healthy control groups [31]. Furthermore, results obtained by Bafadhel et al. (2006) showed that CRP levels did not significantly discriminate between patients with asthma and COPD [16]. This study included 96 adult patients with asthma, 62 adult patients with pneumonia, and 161 adult patients with COPD exacerbations.

However, different results were obtained when the same marker was tested using highly sensitive CRP (hs-CRP) determination. Hs-CRP test essentially quantifies the same marker but is able to detect very small amounts of the protein in serum (0.5–10 mg/L), whereas normal CRP quantification is only able to measure in the range from 10 to 1000 mg/L. In a study comprising 24 corticosteroid naive adults with asthma, 26 adults with asthma treated with corticosteroids and 15 healthy controls of similar age, Allam et al. (2009) showed that hs-CRP is significantly increased in patients with asthma, even in those treated with corticosteroids, when compared to healthy controls ($p < 0.01$) [32]. Another interesting study involving hs-CRP was performed by Wood et al. (2012), showing that hs-CRP is not significantly elevated in every asthma phenotype but only in neutrophilic asthma ($p = 0.011$), which is characteristically more severe, with increased systemic inflammation [33]. A total of 106 adults with non-neutrophilic asthma, 26 adults with neutrophilic asthma and 83 adult controls participated in this study. With these results in mind, together with the simplicity and accessibility of the test, hs-CRP may be a possible serum marker for further supporting a clinical diagnosis of asthma since it can indirectly detect the degree of airway inflammation. However, these results should be interpreted with caution since another study, by Tilemann et al. (2007), contradicted them by showing that hs-CRP levels in asthmatic patients were not significantly different from those in subjects with no OAD, thus suggesting hs-CRP may only be accurate for the diagnosis of COPD [22]. The population of this study included 86 adult patients with asthma, 36 adult patients with COPD, 15 adult patients suffering from diseases that may cause changes in the airways with partial reversibility, and 75 healthy adult controls.

Altogether, hs-CRP appears to be much more clinically reliable as a marker of asthma when compared to ordinary CRP. However, the conflicting evidence around the efficiency of this biomarker may raise some doubts about its usefulness in the diagnosis of asthma [22,32].

Eosinophil cationic protein

Eosinophils are known to play an important role in inflammation in asthma, with increased numbers of this cell type having been reported in peripheral blood and airway secretions in asthmatic patients (for review see [34]). One of the eosinophil granule proteins, eosinophil cationic protein (ECP), has been widely characterized and researched as a marker of asthma [6,35]. In a study involving 441 adult patients with respiratory diseases and 33 healthy adult controls, Peona et al. (2010) showed that ECP was significantly higher in the affected group, compared with healthy

volunteers, particularly in female patients ($p < 0.001$) [36]. In addition, the significantly high ECP levels in asthma compared to other respiratory diseases led the authors to suggest that serum ECP may be considered a marker for identifying patients with isolated asthma with an efficiency of 73%, despite not being useful for the discrimination of other respiratory disorders. However, the authors did not find any correlation between serum ECP and the absolute numbers of peripheral blood eosinophils. Studying 17 adults with asthma before treatment with inhaled steroids, 10 adults with asthma treated with inhaled steroids and 10 healthy adult controls, Ozseker et al. (2006) found similar results in asthmatic patients not treated with inhaled steroids and further showed that there is no significant difference in the expression of ECP between healthy subjects and asthmatic patients treated with inhaled steroids [37]. These findings support the theory that eosinophilic asthma is usually responsive to corticosteroids and that ECP may be a good marker for distinguishing eosinophilic asthma from other OAD and other asthma phenotypes. However, King et al. (2004) showed that ECP levels were not significantly different between asthmatic and non-asthmatic patients in the elderly population, suggesting that ECP is elevated in the elderly population but is not related with asthma [29]. These results could compromise the usefulness of serum ECP as a marker for eosinophilic asthma in elderly. In addition, Koh et al. (2006) state in their review that ECP correlates well with airway inflammation but not with airway hyper-responsiveness and it is increased not only in asthma but also in other atopic diseases, thus not being a good marker for the diagnosis of asthma [38]. Nevertheless, results may support ECP as a guide to tailoring down inhaled corticosteroids.

Soluble CD86

T cells express CD28 and CTLA-4 molecules, which are important for receiving activation signals from antigen presenting cells (APCs), such as monocytes and dendritic cells. These molecules interact with B7 molecules on APCs, B7-1 (CD80) and B7-2, also known as CD86 [39]. Soluble CD86 (sCD86) results from an alternatively spliced transcription characterized by deletion of the transmembrane domain in resting monocytes, and is found in human serum [40].

Since monocytes and dendritic cells play an important role in antigen presentation and activation of inflammatory cells, it has been speculated that sCD86 levels may be altered in patients with asthma [41,42]. Shi et al. (2004) performed a study with a population including 24 adult patients with stable asthma, 28 adults with aggravated asthma, and 25 healthy adult controls in which serum CD86 expression levels were measured in stable patients with asthma, acute patients with asthma, and healthy controls [43]. Levels of serum sCD86 were significantly higher in patients with acute asthma when compared to patients with stable asthma and controls ($p = 0.001$). However, no significant difference was found between CD86 levels in patients with stable asthma and controls ($p = 0.079$). This significant increase in sCD86 in acute asthmatics may be correlated with the Th1-type response featured by this more severe kind of asthma, which explains increased monocyte activity when compared

to the Th2-type asthma, and consequentially the increased levels of sCD86. Should these results be confirmed, sCD86 may become a possible marker of asthma severity.

Serum amyloid A

Just like CRP, serum amyloid A (SAA) is an APP released by the liver in response to various aggressions leading to immune responses [44]. In the previously mentioned study with CRP involving 20 adult patients with asthma and 50 adult patients with rhinitis, along with 20 healthy adult controls, Buyukozturk et al. (2004) also measured SAA and compared the results with the same protein levels in healthy subjects [31]. The results showed significantly increased serum levels of amyloid A in both patients with asthma and patients with rhinitis, compared to healthy controls ($p = 0.002$ and $p = 0.02$, respectively). These results suggest that SAA may be a more useful marker of asthma inflammation than CRP. However, the similar levels of SAA observed in both patients with allergic rhinitis and patients with asthma suggest that this marker does not discriminate between these two types of manifestations of the atopic syndrome [31,45]. Nevertheless, it is crucial to stress that the levels of SAA in patients with asthma, both before and after treatment with Flixotide™ (fluticasone propionate) and Ventolin™ (albuterol) were significantly greater than in healthy controls ($p = 0.038$), in the previously mentioned study performed by Ozseker et al. (2006) [37], which suggests that testing for SAA levels may be sensitive enough to detect asthma inflammation even in patients on inhaled steroid treatment.

IL-8

One of the most studied cytokines in asthma is IL-8 since it is a major chemo-attractant for neutrophils [46,47]. IL-8 is generally correlated with the extent of neutrophilic inflammation and with disease severity. This was shown in a study by Wood et al. (2012), who measured serum IL-8 levels of 26 adult patients with neutrophilic asthma, 106 adult patients with non-neutrophilic asthma, and 83 healthy adult controls [33]. Serum IL-8 was significantly increased in the neutrophilic asthma group when compared to the other two groups ($p < 0.001$), whereas no significant difference was found between non-neutrophilic asthma and healthy controls. These results suggest that IL-8 expression in serum correlates with the levels of neutrophilic inflammation in asthma. Furthermore, they showed that neutrophilic asthma is generally more severe with increased systemic inflammation. This corroborates the results from a prior study performed by Silvestri et al. (2006) that involved 14 adult patients with severe asthma, 22 adult patients with mild/moderate asthma, and 20 healthy adult controls [48]. The results suggest that IL-8 is significantly increased in asthmatic patients when compared to healthy controls ($p < 0.05$ and $p < 0.001$, respectively for mild and severe patients with asthma). Moreover, serum IL-8 levels in patients with severe asthma were significantly higher than in patients with mild/moderate asthma ($p < 0.05$). In short, the results from these two studies suggest that severe asthma is associated with IL-8 and indirectly with neutrophilia. Silvestri et al. also stated that the patients with severe asthma in their study were significantly

older (mean age 56.64, $p < 0.05$) [48], which also supports the notion that elderly asthma is generally more neutrophilic and more severe. Still, Hollander et al. (2007) showed that serum IL-8 levels in asthma overlap with COPD [49]. Thirteen adult patients with asthma and 25 adult patients with COPD participated in this study. As we discussed before, comorbidity is a common feature in the geriatric population and asthma is often misdiagnosed as COPD, which could be a problem when using IL-8 as a marker for elderly asthma.

IL-6

IL-6 is a pro-inflammatory cytokine that stimulates the production of APP by the liver, and is indirectly related to systemic inflammation during asthma exacerbations [50]. IL-6 is also known to be elevated in COPD patients and is associated with lung function decline and systemic inflammation [51]. In the previously discussed study, Wood et al. (2012) analyzed serum levels of IL-6 in patients with neutrophilic asthma, patients with non-neutrophilic asthma, and healthy subjects [33]. IL-6 serum levels were significantly increased in the patients with asthma, when compared to healthy controls ($p < 0.001$). Between the two asthmatic groups, IL-6 levels were significantly higher in the patients with neutrophilic asthma ($p < 0.05$), again suggesting that neutrophilic asthma is generally more severe. Furthermore, they compared these results with those of hs-CRP and concluded that IL-6 is more clinically relevant as a marker of systemic inflammation than hs-CRP.

IL-17

Recent advances in both immunological and clinical phenotyping of asthma have raised the possibility that Th17 cells may trigger the pathology or co-exist with a Th2 type inflammation [52–54]. Th17 cells are T helper lymphocytes that produce elevated levels of IL-17. A recent study found increased numbers of Th17 cells in allergic patients, compared to non-allergic, and further showed that IL-17 could also induce class switching to IgE in human B cells, suggesting a more direct mechanism for Th17-induced atopic phenotypes [55,56]. Further studies in human patients have demonstrated that Th17 cells may also play a role in asthma pathogenesis by promoting airway smooth muscle cell migration, consequently leading to airway re-modelling and possible airway narrowing [55,57]. Agache et al. (2010) showed that serum IL-17 was significantly increased in 36 adult patients with severe asthma, when compared with 49 adult patients with mild and moderate asthma ($p < 0.001$) [58]. These results suggest that IL-17 may turn out to be a good serum marker for measuring asthma severity. Moreover, they concluded that TNF- α was less discriminative in predicting asthma severity, when compared to IL-17. However, serum levels of IL-17 were shown to be inversely related to smoking habits. In other studies, IL-17 in COPD was found to be considerably elevated when compared to asthma, although no association with neutrophilic inflammation was found [54].

TNF- α

Just like IL-6 and IL-17, TNF- α is a pro-inflammatory cytokine indirectly related to systemic inflammation during

asthma exacerbations [30]. Wood et al. (2012) also measured the levels of serum TNF- α in patients with neutrophilic asthma, patients with non-neutrophilic asthma, and healthy subjects [33], showing that no significant difference was found in TNF- α measurement among the three groups ($p=0.247$). Silvestri et al. (2006) showed contradictory results as serum levels of TNF- α were significantly higher in adults with severe asthma than in adults with mild/moderate asthma and healthy adult controls ($p<0.05$ and $p<0.001$, respectively) [48]. This shows that TNF- α can be used as a marker for systemic inflammation in asthma. Unfortunately, there are no studies that can help us to elucidate whether TNF- α levels are affected or not by neutrophilia in individuals with severe asthma.

There are no studies regarding IL-6, IL-7 and TNF- α in the serum of elderly patients with asthma (although Silvestri et al. stated that patients in the group with severe asthma were considerably older), nor studies comparing the same cytokines in asthma and COPD.

Neopterin

Neopterin is a pteridine derivative that is produced by activated macrophages and is a marker of immune activation [59]. Mota-Pinto et al. (2006) performed a study in an elderly population including 42 allergic patients with asthma and 22 non-allergic patients with asthma, along with 41 healthy controls, which allowed them to suggest that serum neopterin is significantly increased in non-allergic patients with asthma when compared to allergic patients with asthma and healthy elderly controls ($p<0.05$) [60]. Non-allergic asthma is generally more Th1-mediated than its allergic counterpart, and the increased neopterin levels may be related to higher macrophage activity, which could explain the results obtained. The results also suggest that serum neopterin may prove to be a useful marker for classifying asthmatic patients, including those in the elderly population [60]. This may be true when classifying asthmatic patients as allergic or non-allergic, but not when attempting to discriminate between asthma and other OAD. COPD, for instance, is also characterized by Th1-type immune responses and serum neopterin levels of patients with COPD may overlap with those from non-allergic asthma. Furthermore, Frick et al. (2004) showed that serum neopterin levels increase naturally with age [61], which could generate doubt about the reason for its increment in elderly patients: are raised serum neopterin levels due to non-allergic asthma inflammation or immunosenescence? However, Mota-Pinto et al. used an elderly population control group, which suggests that neopterin increase in their study was related to non-allergic asthma inflammation.

YKL-40

Chitin is commonly found in nature, as a component of the exoskeletons of crabs, the walls of fungi, or the microbial sheath of parasitic nematodes [62]. Chitin cannot be synthesized by mammals, but they do synthesize chitinases, which can cleave chitin, thus playing a role in defense against chitin-protected pathogens [63]. YKL-40 is a chitinase-like protein (also called chitinase 3-like 1) that lacks chitinase activity.

Chupp et al. (2007) hypothesized that the level of expression of YKL-40 would be increased in patients with asthma and would correlate with the severity of the disease [64]. Therefore, these investigators performed a cross-sectional analysis of serum samples from three cohorts of adult patients with asthma, as well as from healthy adult controls. The results showed that serum YKL-40 levels were significantly higher in patients with asthma compared to healthy controls, in the Yale and Paris cohort ($p=0.02$ and $p=0.002$, respectively). In addition, YKL-40 levels correlated positively with total serum IgE and peripheral blood eosinophils, thereby suggesting that YKL-40 either participates in the pathogenesis of asthma, or is a marker of its severity. Similar results were obtained by Tang et al. (2009), who also reported that YKL-40 levels were significantly higher in 62 adults with asthma when compared to 64 healthy adult controls ($p=0.003$) [65]. Specjalski et al. (2011), showed that YKL-40 levels were significantly higher in 59 adult patients with asthma when compared to 29 healthy adult controls ($p<0.001$), but also stressed that no relationship was found between YKL-40 levels and asthma severity, asthma control, or total serum IgE levels, somehow contradicting the results obtained in other studies [66]. Nevertheless, the authors showed that serum YKL-40 levels correlated with blood eosinophilia ($p=0.05$), thus corroborating the results from Chupp et al. [64,66].

Serum YKL-40 appeared to be a good marker for asthma, but it was not clear whether it was a biomarker of severity or a participant in the pathogenesis of asthma. However, Bara et al. (2012) showed that increased serum YKL-40 levels were positively correlated with bronchial smooth muscle cell migration in 40 adults with asthma ($p<0.05$), suggesting that it may be an active player in bronchial smooth muscle remodeling in asthma [67]. These results potentially link YKL-40 to the pathogenesis of asthma. Nevertheless, YKL-40 levels also appear to be increased in patients with COPD [68], probably because it involves airways remodeling as well. Other diseases such as peripheral arterial disease and diabetes or pre-diabetes also showed increased expression of YKL-40 [69,70]. It should also be stressed that none of the existing studies were focused on elderly patients with asthma, thus making it unclear whether serum YKL-40 levels are affected by aging and immunosenescence.

Leukolysin

Leukolysin is a membrane-type metalloproteinase that appears to be expressed only in granulocytic cells of the myeloid series, mainly in neutrophils, and is released by degranulation or shedding after stimulation with pro-inflammatory cytokines, such as IL-1 and IL-8 [71,72]. Because of this limited distribution in granulocytic cells, Blumenthal et al. (2010) performed a study to evaluate the role of leukolysin in chronic inflammatory conditions, including atopic asthma and COPD [73]. The results showed that serum leukolysin levels correlated well with total serum IgE levels in patients with both atopic asthma and COPD ($p<0.001$ and $p=0.024$, respectively). The population consisted in 337 patients from nuclear families with asthma (including children and adults), and 122 “married-in” individuals.

No study on the behavior of leukolysin in elderly asthma was performed, which means there is no information about how aging affects this MMP expression in inflammatory granulocytes.

Discussion

As convenient as serum markers may seem to be, a marker capable of accurately identifying asthma with sufficient specificity is yet to be found. Furthermore, all the aforementioned markers have scarcely been studied in elderly patients. For instance, studies on the accuracy of sCD86 in elderly asthma are lacking and it is also necessary to clarify whether the significant increase observed in this marker in patients with severe asthma is related to the disease itself. Neopterin and leukolysin undergo similar limitations.

When evaluating IgE as a marker of asthma, specific IgE appears to be more clinically relevant than total IgE for differentiating between atopic or non-atopic asthmatics. However, both specific and total IgE quantification may lose accuracy due to abnormal IgE levels produced in response to other infections. Furthermore, in elderly patients, this marker can be even less accurate since its levels are usually reduced due to natural immunosenescence [15]. The same may happen in patients under pharmacotherapy, which may induce changes in serum IgE levels, thereby compromising the accuracy of the tests [74]. Moreover, because elevated serum IgE levels are not an accurate discriminator of atopic asthma (especially in the geriatric population), leukolysin may indirectly share this inaccuracy as well, since changes in leukolysin levels are closely paralleled by those of total IgE in patients with asthma. However, if good results are obtained in subsequent studies, leukolysin may prove to be a metabolic marker associated with atopic asthma and atopically sensitized COPD patients that can be measured in human serum [73].

Hs-CRP and SAA appear to be more clinically reliable as markers of airways inflammation than ordinary CRP measurement, as they are able to indirectly detect asthma exacerbations. Nevertheless, they may also be responsive to COPD, pneumonia and other forms of airway inflammation-causing diseases. This overlap may compromise their accuracy as specific non-cytokine markers of asthma but might become important as routinely used supplemental indicators of the severity of the disease. However, no studies have analyzed the impact of age on SAA and hs-CRP in elderly patients with asthma.

Currently, IL-8 seems to be a promising cytokine for measuring asthma severity, even in elderly asthma, although it cannot accurately distinguish between asthma and COPD. Studies including a more extensive population are also welcome. Still, it appears to be more clinically reliable than IL-6, TNF- α and IL-17 as severity markers of asthma.

ECP seems to be an interesting protein to be measured when researching for biomarkers of asthma. However, its accuracy is somehow questionable as each study presents slightly different results and its diagnostic value can vary with the age, gender and treatment patients are on.

YKL-40 positively correlates with blood eosinophilia and bronchial smooth muscle remodeling, thus appearing to be an

emerging marker for asthma, that is not only related to asthma exacerbations, but also to the airways remodeling characteristic of the disease.

Conclusion

Altogether, clinically assessable markers capable of supporting a diagnosis of asthma are needed in order to counter mistreatment and misdiagnosis with other OAD, having in mind both the convenience and quality of information they provide [75]. It is essential that these novel markers are capable of diagnosing specific asthma phenotypes. This necessity becomes even more important in the case of elderly asthmatic patients, in whom morbidity is higher and symptoms often overlap with those of COPD [3]. In addition, the presence of some biomarkers may be associated with diseases that do not affect the airways; therefore finding a marker that can differentiate between OADs is not enough. The actual process of aging may, on its own, make a diagnosis of asthma more complicated due to immunosenescence and natural changes in airways morphology, which may compromise the expression of some asthma markers. To develop an accurate marker of asthma, one must make sure that these conditions are taken into account and controlled for, as much as possible.

Declaration of interest

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