UNIVERSITÀ DI PARMA

UNIVERSITA' DEGLI STUDI DI PARMA

DOTTORATO DI RICERCA IN SCIENZE MEDICHE

CICLO XXXII

ENVIRONMENTAL AND OCCUPATIONAL EXPOSURES IN EOSINOPHILIC GRANULOMATOSIS WITH POLYANGIITIS. A CASE-CONTROL STUDY

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Anni 2016/2018

SUMMARY

ABSTRACT

Background and aims of the study

Eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss Syndrome) is a rare systemic disease, included in the group of antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV), characterized by adult-onset asthma, blood and tissue eosinophilia with organ involvement, and small-vessel vasculitis. The disease pathogenesis is poorly understood. Immunogenetic factors may predispose to the disease. However, it has been suspected that the disease may be triggered by exogenous factors including environmental agents, infections, vaccinations, and drugs. Data about the association between individual, environmental and occupational risk factors and the development of EGPA are scarce. In this study we aimed to investigate the role of occupational agents (such as silica, farming, organic solvents and chemical agents) as well as individual agents (such as smoking habits) and their interactions, as potential risk factors for EGPA.

Patients and methods

The study has a case-control design. We enrolled 111 patients with EGPA and 333 healthy controls, matched for age, sex and geographical origin. Occupational history was obtained using a structured questionnaire administered by blinded specialists in occupational medicine. The exposures to non-occupational risk factors potentially predisposing to EGPA were assessed through the interview and through the examination of medical records. All exposures were considered until the time of EGPA diagnosis.

Results

EGPA was associated with silica exposure (OR 2.26 [95% CI 1.10-4.62], p=0.026), farming (OR 2.10 [95% CI 1.19-3.73], p=0.011) and with the exposure to organic solvents (OR 2.20 [95% CI 1.14-4.2], p=0.018) at a multivariate analysis. There was a positive relationship between the duration of exposure to silica, chemical agents and the risk of developing EGPA. A multiplicative effect on risk was found for the co-exposure of silica and farming (OR 7.49 [95% CI 2.77-20.25], p<0.0001). The exposure to tobacco smoke appeared protective against EGPA (OR 0.49 [95% CI 0.29-0.70], p<0.0001).

Conclusions

This is, to our knowledge, the first study investigating the role of environmental, occupational and individual risk factors in the development of EGPA, through the comparison of a group of EGPA patients with a group of healthy controls, matched for age, gender and geographical origin. Our results demonstrate that occupational factors may significantly contribute to the development of the disease. In particular, we found a significative association between exposure to silica, farming, organic solvents and EGPA. In addition, our findings reveal that tobacco smoking has a protective role against the disease. Further studies are necessary to investigate the biological effects of such risk factors on disease pathogenesis.

INTRODUCTION

In 1951, Churg and Strauss first described a syndrome characterized by adult-onset asthma, blood and tissue eosinophilia with organ involvement, and small-vessel vasculitis (SVV) [1, 2]. In their paper, Churg and Strauss described the histology of 13 severe cases, some of which from autopsies: in most organs, they found tissue eosinophilia, necrotizing and granulomatous vascular lesions and extravascular granulomas. These features identified a syndrome that could be distinguished from classical polyarteritis nodosa and also from granulomatosis with polyangiitis (Wegener's, GPA) and it was named Churg–Strauss syndrome for many years [1]. The identification of antineutrophil cytoplasmic antibodies (ANCA) in nearly 30-40% of patients resulted in the inclusion of Churg-Strauss syndrome in the ANCA-associated vasculitides (AAVs), a group of rare immune-mediated diseases which also include GPA and microscopic polyangiitis (MPA) [3]. Finally, in 2012, the revised nomenclature for vasculitides recognized this entity as eosinophilic granulomatosis with polyangiitis (EGPA) [4].

Classification criteria and definitions for EGPA

There are no commonly accepted diagnostic criteria for EGPA [5]. In 1984, Lanham et al. proposed that patients with EGPA should have asthma, eosinophilia, and vasculitic involvement of two or more organs [6]. In 1990, the American College of Rheumatology (ACR) developed the classification criteria for vasculitis, that allowed the distinction between the syndromes, in the presence of histologically-proven vasculitis [7]. EGPA criteria were asthma, eosinophilia >10%, neuropathy, non-fixed lung infiltrates, paranasal sinus abnormalities and extravascular eosinophils on biopsy. When four or more of these criteria are met, vasculitis can be classified as EGPA with a sensitivity of 85% and a specificity of 99.7%. In 1993, the Chapel Hill consensus conference (CHCC) produced mutually

exclusive clinico-pathological definitions for primary vasculitides and described EGPA as a syndrome characterized by eosinophil-rich and granulomatous inflammation involving the respiratory tract, necrotizing vasculitis affecting small to medium-sized vessels, associated with asthma and eosinophilia [3]. The CHCC 2012 replaced the former eponym (Churg-Strauss syndrome) with EGPA and classified the disease among AAVs, even if it is clinically distinct from GPA and MPA and shows ANCA in only 30-40% of patients, compared with the 70-90% of ANCA positivity of patients with GPA and MPA [4].

In 2007, the European Medicine Agency (EMEA) introduced an algorithm for the classification of AAV and polyarteritis nodosa (PAN), that allowed the application of ACR criteria in patients with a clinical diagnosis of vasculitis, based on results of clinical, laboratory and imaging investigations and not only on histological findings [8]. This algorithm also introduced the concept of surrogate markers of vasculitis, i.e. clinical, laboratory or imaging findings highly suggestive of specific vasculitis syndromes. The EMEA algorithm appears useful to diagnose and classify EGPA cases based on the clinical presentation and the results of non-invasive tests, considering that obtaining diagnostic samples is not always feasible and vasculitis not always evident in these patients.

Epidemiology

Classification criteria and their application deeply influence the results of epidemiologic studies, especially on rare diseases such as EGPA. The annual incidence of EGPA has been reported to be 0.5–4.2 cases/10^6 inhabitants with a prevalence of 14-18 cases/10^6 inhabitants [9, 10]. EGPA usually arises in people aged 40–60 years, but paediatric cases have also been reported [11]. No gender predominance or ethnic predisposition have clearly been demonstrated [12].

Genetic and triggering factors

Many studies demonstrated that immunogenetic factors confer susceptibility for EGPA. It has been proven that this condition is associated with HLA-DRB1*04 and *07 and with HLA-DRB4, suggesting an allergen- or antigen-driven process that leads to T cell activation [13]. Interestingly, the research of single nucleotide polymorphisms (SNP) of the gene that encodes interleukin (IL)-10, an important molecule for the activation of the Th-2 pathway, has demonstrated an association between the ANCA-negative EGPA subset and the IL10.2 haplotype of the IL-10 promoter gene [14]. This condition leads to an increased production of IL-10 and is apparently in line with EGPA pathogenesis, which is characterized by an increased Th-2 response and an increase in IgG4 levels, both mediated by IL-10. Recently, based on the evidence that low copy numbers of the gene encoding Fcg receptor 3B (FCGR3B) predispose to systemic autoimmune diseases, we investigated whether FCGR3B copy number variations (CNVs) confer susceptibility to EGPA [15]. The results of the case-control study including 126 patients, revealed that FCGR3B deficiency might predispose to EGPA, particularly the vasculitic disease subset, and might contribute to disease pathogenesis through a delayed clearance of immune complexes by neutrophils and the promotion of a proinflammatory status. However, the ongoing genome-wide association studies will better clarify the genetic susceptibility patterns of EGPA and its sub-phenotypes.

EGPA was long suspected to be triggered by exogenous factors including environmental agents, infections, vaccinations, and drugs. To date, neither infectious agents nor vaccinations have been identified as disease triggers. Silica has been reported as a moderate risk factor for all AAV types, but results were not yet confirmed by further studies [16]. Numerous studies have described the development of EGPA following treatment with different drugs, particularly anti-asthmatic agents, such as leukotriene receptor antagonists (LTRAs) and omalizumab [17-19]. However, a direct pathogenetic role of LTRAs in EGPA has not been yet demonstrated and the relationship between

LTRAs (and the anti-IgE agent omalizumab) and EGPA could be explained by the steroid-tapering allowed by these drugs during asthma therapy. Two case-control studies demonstrated that the risk of EGPA onset was not increased by LTRA exposure [18, 20]; on the other hand, a recent monocentric study found that ANCA+ patients are significantly associated with LTRA treatment, suggesting that leukotriene blockade may influence the development of ANCAs and their related features [21].

Clinical manifestations and laboratory findings

EGPA is a systemic disease that progresses through three different stages, namely prodromal, eosinophilic and vasculitic [6, 22]. The prodromal phase is characterized by asthma and rhino-sinusitis. After a variable period of time (usually 8-10 years), the patients may develop the eosinophilic phase which is hallmarked by peripheral eosinophilia and organ involvement. In the vasculitic phase, instead, the patients show clinical manifestations due to small-vessel vasculitis. These phases partially overlap and may not appear in such a defined order, although asthma and rhino-sinusitis only rarely arise after the vasculitic manifestations.

Asthma is found in 95–100% of patients and may precede the systemic disease manifestations of many years [22-24]. It generally arises in adulthood, and its severity varies; unlike the classical bronchial asthma, in EGPA, asthma does not show the typical seasonal exacerbations and patients have negative tests for common allergens. Moreover, patients are also affected by recurrent rhinitis and sinusitis [25, 26]; nasal polyps affect ~50% of the patients and systematically recur after surgery in patients not receiving immunosuppressive therapy. Other otolaryngologic manifestations include otitis media, chronic ear drainage, sensorineural hearing loss, and facial nerve palsy.

The eosinophilic phase is characterized by lung, cardiac, and gastrointestinal (GI) involvement. Lung infiltrates are among the most typical EGPA manifestations, occurring in 40-60% of the patients [22].

Chest X-ray abnormalities generally consist of mainly peripheral, patchy, and migratory infiltrates. High-resolution computed tomography (HRCT) accurately detects further interstitial lung lesions, such as non-cavitating nodules and bronchial thickening [27]. Alveolar haemorrhage is rarer than other AAVs, affecting 3–8% of the patients [28].

Heart involvement is the first disease-related cause of death and affects about one-fifth of the patients [29]. All heart structures may be involved but the main patterns are endo-myocarditis and pericarditis with pericardial effusion. A study on 49 patients reported symptoms of acute or chronic heart failure and significant abnormalities at echocardiography/electrocardiography in 22 patients (45%) [30]. However, cardiac magnetic resonance imaging (cMRI) with late-gadolinium enhancement technique appears the most sensitive imaging study, able to detect active myocarditis and fibrosis. Recently, it has been demonstrated that a high proportion of asymptomatic patients have cMRI abnormalities, yet of unclear clinical and prognostic significance [31, 32].

The GI tract is less frequently involved and main manifestations are non-specific abdominal pain, diarrhoea and minor bleeding, often due to eosinophilic infiltration of the mucosa [33]. The disease more frequently affects the small bowel and may cause severe manifestations, potentially requiring surgery, in 22-45% of cases, such as bowel perforation and ileum [34]. In rare cases, acalculous cholecystitis or pancreatitis may represent the dominant manifestations [35].

The vasculitic phase is heralded by constitutional symptoms (i.e. fever, weight loss, fatigue) and often by an apparently paradoxical improvement of asthma. In this phase peripheral nervous system is frequently involved, with a sensory-motor peripheral neuropathy which affects more than 50% of the patients [36]. Electroneurography shows alterations compatible with axonal damage. Typically, symptoms are referred in asymmetric, separate nerve areas, a pattern called *mononeuritis multiplex* [22].

Renal manifestations are rarer than in GPA and MPA, as they are found in up to 25% of the patients, and range from isolated urinary abnormalities (i.e., microscopic haematuria, proteinuria) to rapidly progressive glomerulonephritis [37]. Palpable purpura is the main cutaneous manifestation. It occurs in about the 25% of cases and usually involves the lower limbs. However, more severe cutaneous manifestations, such as nodules, urticaria, livedo, and skin ulcers are also reported [22]. Patients with EGPA show an increased risk of venous thrombo-embolic events, such as deep venous thrombosis and/or pulmonary embolism, probably due to specific disease factors which contribute to hypercoagulability, such as eosinophil-related endothelial damage [38].

Finally, rare cases of EGPA overlapping with IgG4-related diseases have been described [39]. Patients may present with tumour-like masses located in retroperitoneum, pancreas or salivary gland and histology can reveal findings consistent with IgG4-related disease (IgG4-RD), including storiform fibrosis, obliterative phlebitis and lympho-monocytic infiltrates rich in IgG4+ plasma cells [40]. Notably, IgG4 levels are elevated also in active EGPA, thus underlining a possible link between the two conditions.

Active EGPA is characterized by marked peripheral eosinophilia (usually >1500 cells/µl or >10%). It correlates with disease activity, and increases when disease relapses. C-reactive protein and erythrocyte sedimentation rate (ESR) are also usually elevated in the active phase. IgE, usually tested for asthma evaluation, are frequently high, although common allergen specificity at radioallergosorbent tests (RAST) is low [41]. Intriguingly, it has been shown that serum IgG4 and IgG4/IgG ratio are high in 75% of active EGPA patients and that they correlate with disease activity better than IgE do [42].

ANCA are positive in 30-40% patients and mostly have specificity for myeloperoxidase (MPO) at ELISA [22, 43, 44]. Although there are no validated diagnostic tests for EGPA, new biomarkers are emerging. A recent study demonstrated a good diagnostic performance of eotaxin-3, a eosinophil-

attracting chemokine; its serum levels were significantly higher in active EGPA than in a wide range of control groups, including other AAVs and hypereosinophilic syndromes (HESs) [45, 46]. However, further studies are needed in order to confirm the diagnostic value of this biomarker.

Disease subsets

The clinical phenotype of EGPA is varied. However, its clinical manifestations tend to segregate into two major disease subsets differentiated by ANCA status. It has been demonstrated that ANCA+ patients have higher odds of vasculitic manifestations, *i.e.* glomerulonephritis, skin purpura, and peripheral neuropathy, while ANCA-negative patients have more frequently "eosinophilic" manifestations such as cardiomyopathy, gastro-intestinal involvement and lung infiltrates [43, 44]. This distinction raises the question as to whether ANCA contribute to the development of vasculitis in EGPA and if this influences the prognosis of the disease. A case series published by the French Vasculitis Study Group in 2013, reported that ANCA-positive patients are more prone to relapse, while ANCA-negative patients carry a higher risk of death, suggesting that treatment may have to be adapted according to ANCA status [22].

Pathogenesis

EGPA shows a heterogeneous clinical and pathological phenotype that suggests a profound immune dysregulation. It has been demonstrated that both Th1 and Th2 pathways are activated, while eosinophils are responsible for most tissue damage [47-49]. T-lymphocytes have an important role in EGPA pathogenesis. They are present in most organ lesions, representing the main inflammatory component in some of them. Serum levels of T-cell activation markers, like IL-2r, are increased during the active phase of the disease and T-cells receptors show a restricted repertoire suggesting oligoclonal expansion, that is in line with the hypothesis of an antigen-driven disease.

EGPA is classically considered a Th2-mediated disease. In keeping with this view, it has been demonstrated that EGPA patients CD4+ T-cells are able to produce, *in vitro*, high concentrations of IL-4, IL-5, and IL-13, molecules that hallmark the Th-2 immuno-response. The up-regulation of IL-5 is also evidenced by the beneficial effects of IL-5 inhibition through mepolizumab in EGPA patients. Paranasal sinus biopsies of patients with EGPA are rich in T-cells with Th-2 makers such as CD294 while cells obtained from bronco-alveolar lavage (BAL) fluid of patients with active disease show a Th2-skewed functional profile, given their high transcript levels of Th2 cytokines (particularly IL-4, IL-5, IL-10). However, the clinical phenotype of EGPA cannot be explained by an exaggerated Th2 response alone. There is evidence of involvement of Th1 and also Th17 cells in the pathogenesis of the disease, by the secretion of high amounts of IL-17A in the late EGPA phases [50]. Finally, lower levels of regulatory CD4+ T-cells have been found in EGPA patients with active than in those with inactive disease [51].

Eosinophils are abundant both in the periphery and in EGPA lesions. Activated tissue eosinophils secrete considerable amounts of eosinophil granule proteins (i.e. eosinophil basic protein, eosinophil-derived neurotoxin), contributing to tissue damage, and IL-25 which induces Th2 responses and thereby maintain a vicious circle.

Chemotaxis is a crucial mechanism to induce tissue damage by immune cells. The chemokine eotaxin-3 is able to attract eosinophils in the sites of inflammation [46]. Its serum concentrations are increased in active EGPA patients and correlate with eosinophil counts, total immunoglobulin E (IgE) levels, and acute-phase parameters. Immunohistochemical analysis of tissue biopsies of patients with active disease also revealed a high eotaxin-3 expression by endothelial and inflammatory cells [45]. The CCL17 chemokine is also detectable both in tissues infiltrates and patients' serum and mediates recruitment of Th2 cells [52, 53].

Recent evidences have highlighted the role of the humoral immunity in EGPA. B cells are precursors of (ANCA-producing) plasma cells but can also present antigens to Th cells, inducing their activation. In addition, Th-2 cytokines enhance the isotypic switch toward IgG4 production [42]. These antibodies are not efficient at activating complement pathways and, for this reason, their pathogenetic role is not clear. Finally, the efficacy of B-cell depletion by rituximab reported in some case series also seems to confirm the contribution of B-lymphocytes to the EGPA pathogenesis [54, 55].

Pathology

The pathology of EGPA varies considerably depending on the site, the type and the stage of the lesions. The main histological features of EGPA are tissue eosinophilia, vasculitis of medium and small vessels, and extravascular granuloma [1, 2]. Vasculitis is characterized by fibrinoid necrosis and eosinophilic vessel wall infiltration. Granulomas may involve the arteries, but the more EGPA specific lesion is the extravascular granuloma, which consists of a core of necrotic eosinophilic material surrounded by lymphocytes and epithelioid and multinucleated giant cells [7]. While lung lesions may show all the aforementioned features, cardiac pathology usually shows endo-myocardial and pericardial eosinophilic infiltration and only rarely coronary vasculitis. Gastrointestinal involvement histologically shows eosinophilic gastroenteritis and, in some cases, mesenteric vasculitis leading to bowel ischemia [34]; conversely, kidney biopsy typically shows focal crescentic glomerulonephritis, while eosinophil infiltrates are rare and granulomas absent [37]. Peripheral neuropathy is due to epineural lymphocytic vasculitis with rare eosinophils, while purpura is caused by leukocytoclastic vasculitis of dermal vessels and usually lacks granulomatous or eosinophilic reactions.

Differential diagnosis

The differential diagnosis of EGPA essentially includes eosinophilic and vasculitic disorders. Parasitic infections as well as hypersensitivity reactions (e.g., to drugs) must be excluded [56, 57]. Eosinophilia is common among atopic patients with asthma, but in these cases, eosinophil counts seldom exceed 1,500/μL. Moreover, eosinophilia and respiratory symptoms are the major features of allergic bronco-pulmonary aspergillosis (ABPA) [58]. The isolation of *Aspergillus spp*. on BAL or sputum and high serum levels of *Aspergillus fumigatus*-specific IgE are diagnostic of this condition. Acute eosinophilic pneumonia is featured by pulmonary infiltrates and BAL fluid rich in eosinophils but usually patients show also an acute illness with fever and dyspnea and do not present with eosinophilic involvement of other organs. On the other hand, EGPA limited to the upper and lower airway tract is difficult to differentiate from chronic eosinophilic pneumonia, because both conditions share a history of asthma, sinusitis and lung infiltrates [59].

Particularly challenging is the distinction between ANCA-negative EGPA and hypereosinophilic syndromes (HESs). The HESs are characterized by persistent and pronounced eosinophilia (generally >1500/μl) and organ involvement, in absence of other causes which may determine eosinophilia (parasitic and viral infections, drugs, allergy, neoplasms, autoimmune, or immune-mediated diseases) [60]. Pathogenesis-driven classification of the HESs subdivides them into four main groups: myeloproliferative and lymphocytic forms associated with abnormalities of PDGFRA, PDGFRB or FGFR1 genes; chronic eosinophilic leukemia or other myeloid neoplasms associated with eosinophilia that lack recognized genetic abnormalities; lymphocytic variant of HES (characterized by a lymphocyte clone abnormally producing IL-5 or IL-3); idiopathic HES [61]. HES may substantially overlap with EGPA: cardiac and pulmonary manifestations may be similar, while HES patients rarely have asthma or polyps and vasculitic complications [62]. HES does not show vasculitis on tissue biopsies and ANCA are typically negative. The differentiation between HES and ANCA-negative EGPA

requires a careful hematologic work-up, aimed to assess B or T cell clonal restriction and to search for *PDGFRA*, *PDGFRB*, *FGFR1*, *BCR/ABL1* fusion genes or Janus kinase-2 mutations.

EGPA must be differentiated also from other AAVs. GPA and MPA usually lack asthma and eosinophilia. Moreover, GPA often shows fixed cavitating lung nodules, paranasal sinus bone erosions and (generally) ANCA specificity for proteinase-3. MPA patients, although usually are P-ANCA/MPO-ANCA as EGPA patients, are typically older than EGPA patients and usually have severe renal involvement.

Finally, given the observation of high serum IgG4 levels in EGPA patients, the disease should be differentiated from IgG4-related disease (IgG4-RD) which may present with allergic manifestations, eosinophilia, lung infiltrates and sinusitis [42]. However, tissue biopsies of IgG4-RD show storiform fibrosis and inflammatory infiltrates, while vasculitis and eosinophilic granulomas are absent [40].

Treatment and outcome

The treatment of EGPA is stratified according to patient prognosis, as assessed by the Five Factor Score (FFS), and the severity of disease manifestations. The FFS, revised in 2011, assigns one point to each of following items: heart involvement, severe GI manifestations, stabilized serum creatinine peak >1.7 mg/dL, age ≥65 years and absence of ENT involvement [63]. Immunosuppressive agents combined with glucocorticoids are administered to patients with one or more predictors of poor prognosis (FFS≥1) or to those patients with organ- or life-threatening manifestations, such as severe peripheral neuropathy or diffuse alveolar haemorrhage. On the contrary, EGPA without poor prognosis score (FFS=0) are treated with glucocorticoids alone [64].

The Birmingham Vasculitis Activity Score (BVAS) assesses disease activity. Disease remission is defined as absence of clinical manifestations consistent with active disease (BVAS=0), excluding asthma and sinusitis exacerbations under daily prednisone dose ≤7.5 mg.

Glucocorticoids are the mainstay of EGPA treatment. The recommended daily dosage at induction is 1 mg/kg of prednisone followed by gradual reduction until withdrawal or ≤7.5 mg/d to limit adverse effects. Induction therapy of patients with poor prognosis includes the association of cyclophosphamide (CYC) and steroids. Courses of 6 to 12 intravenous pulses (15 mg/kg) lead to remission in ≥85% cases, but the risk of relapse is lower with prolonged treatment [65-67]. After induction therapy, maintenance treatment is required in order to maintain remission. CYC is then switched to less toxic drugs, usually azathioprine (2 mg/kg/d) or methotrexate (0.3 mg/kg/week) [68, 69].

Other types of treatment may be considered in specific populations. Due to its lower toxicity compared to cyclophosphamide, methotrexate has also been used for induction, successfully. However, the relapse frequency in long-term follow up of patients treated with this immunosuppressive agent is high. Plasma exchange can favour remission in diffuse alveolar haemorrhage or severe glomerulonephritis [70] while interferon-alpha therapy has been tried with positive results in refractory patients, but the severe drug-related toxicity has limited its use [71].

B-cell depletion with rituximab (RTX) has been reported by retrospective studies as effective in the treatment of ANCA-positive EGPA patients or in EGPA patients with renal involvement [54, 55, 72]. Two randomized controlled trials (RCTs) exploring the role of RTX in this disease are still ongoing. REOVAS compares RTX with standard induction regimens based on the FFS (ClinicalTrials.gov Identifier: NCT02807103), while MAINRITSEG compares scheduled RTX administration with AZA for maintenance (ClinicalTrials.gov Identifier: NCT0316447).

Omalizumab is a humanised anti-IgE monoclonal antibody licensed for the treatment of severe forms of asthma or other allergic syndromes. It prevents IgE-mediated degranulation of eosinophils and may also cause eosinophil apoptosis. Two small EGPA patient series reported that this drug is able to reduce glucocorticoids doses [73, 74]. However, a complete response based on symptoms

and pulmonary function test (PFT) results was observed in only 35-55% and the steroid reduction probably caused a high relapse rate.

Mepolizumab, a monoclonal antibody that prevents the binding of IL-5 to its receptor, licensed for refractory asthma and idiopathic HES, was reported to be effective in some case series including EGPA patients with steroid-dependent asthma [75, 76]. On this basis, Wechsler et al. conducted a double-blind RCT comparing mepolizumab at a monthly dose of 300 mg subcutaneously (thrice the dosage approved for asthma) for one year and placebo in relapsing/refractory EGPA patients treated with standard of care [77]. Mepolizumab appeared effective in prolonging the period of remission of the disease, allowing steroid reduction. The positive results of this study, which met both of the primary endpoints, led to the approval of mepolizumab in adult patients with EGPA by the Food and Drug Administration in 2017. Therefore, mepolizumab can be officially considered as an add-on therapy with steroid-sparing effect in cases of relapsing or refractory EGPA. However, the most appropriate dose and duration of therapy still need to be determined and future studies on larger multinational populations with prolonged follow-up are warranted. In addition, its efficacy on vasculitic disease manifestations remains uncertain.

Finally, two further monoclonal antibodies targeting IL-5 axis, benralizumab and reslizumab, are under investigation in EGPA patients; moreover, anti-IL-13 and anti-IL-4 agents, which are currently being tested in asthmatic patients, might in the future represent possible strategies for EGPA.

BACKGROUND AND AIMS OF THE STUDY

AAVs are rare conditions, and their etiology is poorly understood. Genetic, infectious and environmental factors may influence the risk of development of these diseases. The genome-wide association studies (GWASs) performed in patients with GPA and MPA have demonstrated several genetic variants associated with these conditions [78, 79]. The single nucleotide polymorphisms (SNPs) reported in association with GPA involve the HLA-DP locus, genes encoding autoimmunity-related molecules such as PTPN22 and CTLA4 and others encoding alpha-1 anti-trypsin and proteinase 3 (PR3), the predominant antigenic target of ANCA in GPA [80]. MPA is instead associated mainly with HLA-DQ variants [81, 82]. Interestingly, these associations were stronger with ANCA specificities [PR3-ANCA vs. myeloperoxidase (MPO)-ANCA] than with the clinical syndromes. A GWAS of EGPA has recently been completed, with preliminary results showing both HLA- and non-HLA associations, which may confirm the associations with immune gene variants previously reported by candidate gene studies.

Many reports suggest that, besides genetic polymorphisms, environmental and occupational factors play an important role in the pathogenesis of these diseases [83]. Case-control studies aimed to investigate the association between AAVs and occupational or environmental studies have already been performed. However, these studies are underpowered and their small sample sizes have substantially reduced the reliability of their results. In addition, they only include GPA and MPA patients or include only very small numbers of EGPA patients; for this reason, occupational and environmental risk factors for EGPA warrant investigation.

Silica dust exposure has been associated with a variety of autoimmune disorders, including scleroderma, systemic lupus erythematosus (SLE) and AAV. The first reports on silica exposure in AAV (particularly GPA and MPA with kidney involvement) were published in the 1990s [84-86]. In

most patients with AAV following silica exposure, the target antigen of serum ANCA was MPO, whereas PR3-ANCA was present only in few cases. Wichmann et al. found that individuals chronically exposed to silica, whether or not affected by connective tissue diseases, had levels of anti-MPO antibodies greater than those found in the normal population (27% of silica-exposed subjects) [84]. In 2001 Hogan et al. performed a case-control study including AAVs and lupus nephritis patients that evaluated the association between these conditions and silica exposure. The study enrolled 65 AAV patients (both GPA and MPA) and found that the odds ratio of silica dust exposure was 4.4 for patients with AAV compared with control subjects, without significant differences between patients with or without lung or sinus involvement [87]. In a study including only ANCA-positive AAV patients (a total of 60 patients, including only four patients with EGPA), Beaudreuil et al. found that ANCA-positive patients were significantly more often exposed to silica than control subjects [88]. A recent case-control study including 129 GPA and MPA patients evaluated occupational silica exposure by using an exposure rating that incorporated the intensity, duration, and probability of exposure. The association was most notable from agricultural silica exposure through harvesting of crops. In addition, the duration of exposure represented the primary aspect of silica exposure as risk factor for the development of the diseases [89]. A meta-analysis including six case-control studies, aimed to examine the association of silica exposure and AAVs and to determine the strength of the association between this risk factor and the diseases, concluded that, despite heterogeneity among the different studies, the totality of the evidence points to a strong association between silica exposure and AAVs, without distinction between GPA and MPA [90].

On the basis of the scarce data regarding the association between AAVs and other environmental and occupational risk factors [91, 92], Lane et al performed a case-control study analyzing not only silica exposure but also solvents, metals and farming as possible risk factors for AAV development

[16]. In this paper, published in 2003, the authors included 75 AAVs (16 of them were EGPA patients) and 273 controls (which included subjects with other diseases, i.e. secondary vasculitis or asthmatic patients). They identified, for the first time, an association between AAVs and farming and confirmed that these diseases are associated with silica and solvent exposures. Particularly, a subanalysis performed for each AAV type, revealed that EGPA, unlike GPA and MPA, was only associated with silica while other occupational exposures did not prove risk factors for the disease. More recently, Willeke et al. reported the results of a larger case-control study including 189 AAV patients (22 of them were EGPA) and 190 controls where they investigated the association between farming and AAV. The study demonstrated an association between AAV and farm exposure, but the analysis of the three different groups of disease only showed a significant association with GPA, while no significant association was detected between farming and MPA or EGPA, although it must be acknowledged that the single AAV subgroups were small [93].

Data about asbestos exposure and AAVs are scarce and controversial. Stratta et al. noted that asbestos exposure did not occur more frequently in 31 cases with biopsy-proven AAV than in 58 controls [94]. In 2005, a study including AAV patients with lung involvement reported that 10% of patients had an occupational asbestos exposure, compared with none in the control group [95]. Another study, investigating ANCA positivity in subjects previously exposed to asbestos versus subjects without exposure, showed that ANCAs were more frequently positive in the exposed group. However, there was no difference in AAV incidence among the two groups [96]. The association between AAV and asbestos exposure was not confirmed in larger studies and no data are available on EGPA patients [88].

Few studies analyzed the possible association between smoking habits and AAV. Two studies specifically performed to investigate environmental factors failed to show any association between smoking status and AAV [16, 88]. In a retrospective study performed by Haubitz et al., the

prevalence of smoking in 197 patients with AAV was compared with age-specific values for the general population in Germany. The proportion of active smokers in the group of patients was significantly lower than in the entire population (with data being derived from national databases) and the authors concluded that cigarette smoking may be associated with a reduced risk of development AAV [97]. However, this study only included GPA and MPA patients and no data are available about smoking in EGPA. In addition, its results need to be confirmed by controlled studies with a control group.

In the present case-control study, we aim to investigate the association between exposure to a wide array of environmental and occupational agents and the risk of EGPA. We will also analyze the possible associations with other traditional risk factors for vascular diseases such as smoking, hypertension and obesity. Finally, we will evaluate the possible interactions between these environmental, occupational and cardiovascular risk factors.

PATIENTS AND METHODS

This study has a case–control design, with matching of each case with three healthy controls. In order to avoid a high degree of exposure misclassification due to postal questionnaires, we used an interviewer-administered questionnaire. The questionnaire was administered by a specialist in occupational medicine who was not part of the team of physicians following the patients and was blinded to the study group to which the interviewed person belonged (case or control). Case and control participants were identified by the physicians; they received an appointment for the interview which took place in a dedicated meeting room of the Nephrology Department of the University Hospital of Parma, Parma, Italy. In order to limit the recall bias (an overestimation of exposures by patients), neither the questionnaire nor the informed consent sheet disclosed the aims of the study. Indeed, the study was presented to the participants as an epidemiologic survey aimed at assessing various occupational and nonoccupational exposures in different Italian regions. Patients with EGPA were not informed of the possible association between the different risk factors and EGPA during their routine visits. The study was conducted in compliance with the Declaration of Helsinki and was approved by the local ethic committee. All patients and controls provided a written informed consent before participating in the study. Privacy was preserved through the adoption of alphanumerical codes as identifiers.

Cases and controls

All patients with EGPA diagnosed at or referred to the Vasculitis Clinic of the Medicine and Surgery Department of University of Parma between December 2010 and October 2018 were identified from a prospective vasculitis register and were invited to participate. Case notes were reviewed for clinical and laboratory details including ANCA status. EGPA diagnosis had to fulfill the 1990 American

College of Rheumatology criteria and the 1994 or 2012 revised Chapel Hill Consensus Conference definitions. Disease activity was assessed by the Birmingham Vasculitis Activity Score (BVAS) while EGPA prognosis was assessed according to the revised five-factor score (FFS). Patients under the age of 18 at diagnosis or patients with other primary or secondary vasculitis were excluded. In addition, we excluded patients with cognitive impairment as well as severely ill cases, due to a likely reduction in ability to complete the questionnaire accurately.

We identified 130 patients with EGPA, of whom three were excluded because they were under 18 at diagnosis and one was excluded because he was not Italian, one patient had died prior to the start of the study, one patient was affected from dementia, 11 patients had been lost to follow up and were no more contactable, two declined the participation at the study. The remaining 111 patients with EGPA (85.4%) were enrolled (**Figure 1)**.

A total of 340 control participants were invited to participate in the study. Of them, three declined to participate, and four did not attend the appointment for the interview. The remaining 333 control participants (97.9%) were enrolled (**Figure 2)**. They were case-matched for age (±5 years), sex, and geographical origin (North vs. Center-South of Italy) and were recruited among relatives or friends of patients coming to the Nephrology Unit of Parma University Hospital from all over Italy for kidney transplantation. The recruitment of control participants occurred in parallel with that of the patients.

Exposure Assessment

For the collection of data about medical history and occupational and non-occupational exposures, we developed a structured questionnaire with 3 main sections. It was based on a questionnaire we used previously to perform a study aimed to evaluate asbestos and smoking exposures in patients with idiopathic retroperitoneal fibrosis [98]. We adapted the questionnaire for patients with AAV

including additional questions after a literature review. Analysis of occupational exposure was both qualitative and quantitative, including the evaluation of exposure duration as number of years worked. A copy of the full questionnaire may be obtained from the authors on request.

Section 1 of the questionnaire. In this section, we investigated lifestyle and individual risk factors such as smoking or drug use, cardiovascular or other autoimmune diseases. Tobacco smoking was evaluated both as a categorical variable (presence or absence of the habit) and a continuous variable (pack-years index), in order to measure the cumulative smoking exposure. Persons who abstained from smoking for at least 1 year were classified as former smokers. Hypertension was identified in persons who met at least 1 of 3 criteria: physician diagnosis of hypertension, self-reported use of anti-hypertensive drug therapy, or reported values of systolic blood pressure of 140 mmHg or greater and/or diastolic blood pressure of 90 mmHg or greater. Obesity was defined as a body mass index of 30 kg/m2 or greater. Data on established ischemic heart disease, cerebrovascular accidents, and cancer were obtained from the interview (no physical examination or laboratory tests were conducted as part of the study). Ischemic heart disease was present if 1 or more of the following criteria were met: myocardial infarction, silent myocardial infarction (defined by the documentation of characteristic electrocardiography or a recorded physician's diagnosis in a patient with no documented history of myocardial infarction), revascularization procedures, or a clinical diagnosis of angina. The diagnosis of cerebrovascular disease was based on a history of stroke confirmed by imaging or a history of transient ischemic attack. The diagnosis of cancer was based on a history of any malignant neoplasm for which cases or controls underwent surgery and/or chemotherapy and/or radiotherapy. In patients, all data were collected at the time of diagnosis.

Section 2 of the questionnaire. Section 2 focused on exposure to asbestos, silica and farming. Questions about asbestos exposure derived from a specific questionnaire developed and validated by the Italian National Mesothelioma register (ReNaM). Asbestos exposure was classified as "occupational" or "extra-occupational". "Occupational" exposure was further classified as documented, when persons carry out work implying the use of asbestos; probable, if persons work in industrial settings where this risk factor is certainly present but not documented; possible, if asbestos is probably present but not reported in the work environment. "Extraoccupational" exposure included "familiar" contact (persons who lived with a worker assigned to the documented or possible exposure) and "environmental" contact (persons who lived or had lived near industrial sites that used asbestos).

The items analyzed in order to investigate farming were farm exposure, participation in harvesting, exposure to cattle, pigs, horses or poultry. In addition, we requested the subject's history of residence. Participants were asked whether they lived on or directly next to a farm at any time of their life.

Direct questions were asked to ascertain any history of specific job previously reported to be associated with silica exposure and vasculitis, such as coal or mine workers, sandblaster, baker, dental worker, working in the construction industry.

Section 3 of the questionnaire. Section 3 focused on other occupational exposures. The assessment of occupational exposure to organic solvents, metals, other industrial chemicals, and pesticides was essentially based on the Geoparkinson questionnaire, which was used in a recent European study that assessed these risk factors in patients with either Parkinson disease or parkinsonism [99]. In addition, the evaluation of the exposure to textile fibers was based on the questionnaire used in the ICARE study [100], aimed to investigate this occupational risk factor in patients with lung cancer.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows software (version 25.0, Armonk, NY: IBM Corp.). Continuous variables were displayed as means and standard deviations (SD) or as medians and range. Categorical variables are presented as number (n) and percentage (%). The Student's t-test was used to assess differences between means. Differences between categorical variables were analyzed by the chi-square test. Correlation analyses were performed computing Spearman's correlation coefficients. Univariate and multivariate conditional logistic regression models were used to assess the impact of the exposure to risk factors and the development of EGPA. Odds ratios (ORs), expressed by the values of exp(B), were reported with their respective 95% confidence intervals (CIs) and statistical significance. In the multivariate conditional logistic regression model independent variables were entered using a forward selection method. A two-sided *P*-value ≤ 0.05 was considered to indicate statistical significance. No missing values were present in the database.

RESULTS

The main patient characteristics are summarized in **Table 1**. There were no significant differences in age, gender or geographical origin between EGPA patients and controls.

Non-occupational risk factors

The comparison of the prevalence of non-occupational risk factors for EGPA are showed in **Table 2**. Cases participants had a median BMI value significantly lower than controls (24, IQR 22-26 vs 25, IQR 23-28, p=0.001), even if obesity prevalence did not differ between the two groups (6% vs 8%, OR 0.52 [95% CI 0.23-1.21], p=0.12). Patients had also a lower prevalence of ischemic heart disease (3% vs 12%, OR 0.20 [95% CI 0.06-0.67], p=0.009). Conversely, neoplastic diseases appeared significantly more prevalent in the patients than in the controls (11% vs 4%, OR 2.76 [95% CI 1.24-6.17], p=0.013). The prevalence of other clinical variables such as hypertension, diabetes, and cerebrovascular disease was not statistically different.

The analysis of smoking habit showed a statistically significant lower prevalence of smokers+former smokers among cases (34% vs 54%, OR 0.49 [95% CI 0.29-0.70], p<0.001). In smokers and former smokers combined, the cumulative exposure to tobacco smoking was also significantly greater in controls than in EGPA patients (median 18 pack-years [IQR, 9-30] vs 10 pack-years [IQR 5-20]; p=0.014). In order to exclude that the lower prevalence of smokers in the patients' group was due to disease-related respiratory symptoms (mainly asthma), we analyzed smoking exposure in patients before their asthma onset (median age 43 years old, IQR 36-52) and in 222 healthy controls matched for age (median age 45 years old, IQR 38-23, p = 0.5. Again, current+former smokers' prevalence was significantly lower in the patients than in the controls (**table 2**).

Occupational risk factors

The associations between occupational risk factors and EGPA are summarized in **table 3**.

Working in occupations with high silica exposure in any time during working lifetime was associated with significantly increased OR for EGPA (OR 2.79 [95% CI 1.55-5.01], p=0.001). The duration of occupational silica exposure was examined by comparing non-exposed study participants to those below and above the median of exposure duration in years (median duration=20 years) based on the job history. The odds ratios of EGPA associated with duration of occupational silica exposure demonstrated a positive relationship (**Figure 3, top**). Compared to those not exposed, those below the median duration of silica exposure had an OR of 2.28 (95% CI 1.03-5.06) and those above the median duration of silica exposure had an OR of 5.09 (95% CI 2.11- 12.32).

A history of solvent exposure was associated to risk of developing EGPA (OR 3.19 [95% CI 1.91-5.34] p<0.001) , without a positive relationship in the duration of the exposure (data not shown).

There was a significant association between EGPA and occupations defined as having exposure to chemical agents (OR 1.84 [95% CI 1.12-3-03], p=0.016). The analysis of the duration of chemical exposure revealed an association with a long-time exposure (above the median duration of 20 years), with an OR of 3.30 (95% CI 1.62-6.70), p=0.001. No association between chemical exposure and EGPA was demonstrated when we considered exposures lasting less than 20 years (OR 1.34 [95% CI 0.70-2.56], p=0.38), **Figure 3, bottom**.

EGPA was significantly associated with any type of farming exposures, including living near a farm or working in a farm (OR 2.71 [95% CI 1.71-4.29], p<0.001). It was not possible to distinguish between exposure to crops, livestock or individual animal species because most individuals were exposed to more than one type of exposures. We particularly analyzed the association between EGPA and the exposure to pesticides during worktime and there was no significant difference in the frequency of pesticide exposure in patients vs. controls (OR 1.20 [95% CI 0.58-2.49], p=0.62).

Documented exposure to asbestos was not associated to EGPA (OR 0.94 [95% CI 0.26-3.40], p=0.919). We found a statistically significant although weak association between EGPA and the possible/probable occupational asbestos exposure (OR 2.38 [95% CI 1.22-4.63], p=0.011) and between the disease and all kinds of asbestos occupational exposure (OR 1.93 [95% CI 1.05-3.54], p=0.034). When we considered extraoccupational asbestos exposure, we found a significant association with EGPA (OR 2.22 [95% CI 1.34-3.67], p=0.002). This discrepancy does not appear to provide a strong rationale to support an association between asbestos and EGPA.

Finally, there were no significant associations of occupational exposure to metals and textile fibers with EGPA (**Table 3**).

Multivariate conditional logistic regression models were used to confirm the association between smoking, silica, solvent and farming exposures and EGPA. Sex, age and geographical origin had been used as matching variables and were therefore not included in the models. The results of the analyses using the multivariate conditional logistic regression models showed a significant association between EGPA and silica, organic solvents and farming exposure. The analysis also confirmed a significantly lower frequency of smokers in the EGPA group (**Table 4**). The inclusion of other variables, which were significant in the univariate models (ischemic heart disease and cancer), marginally changed the results with little adjustments of OR and the goodness-of-fit variables. Other covariates used in the models (diabetes, and hypertension) were never significant in the multivariate models.

Given the results with silica, organic solvents and farming as independent disease predictors, we further investigated the co-exposure to farming and silica, farming and organic solvents and organic solvents and silica compared with unexposed persons. The exposure to farming alone and silica alone were significant (OR 2.68, [95% CI 1.61-4.45], p=0.0002 and OR 3.26, [95% CI 1.51-7.02], p=0.004, respectively), but their coexposure led to an OR of 7.49 (95% CI 2.77-20.25, p<0.0001),

suggesting a multiplicative (rather than just an additive) effect between these two variables.-(**Figure 4**). We did not find any increase in the OR in the analysis of the co-exposure to farming and organic solvents and to silica and organic solvents (data not shown).

Subanalysis of ANCA status and clinical manifestations

We performed a subanalysis aimed to investigate the association between ANCA status and clinical disease manifestations and the disease risk factors identified through the multivariate analysis (silica, farming and organic solvents). The results are summarized in **table 5**.

ANCA positivity was significantly associated with the exposure to all the risk factors (silica exposure had an OR 3.85 [95% CI 1.93-7.65], p=0.0002, farming exposure had an OR 2.60 [95% CI 1.45-4.67], p=0.002, the exposure to organic solvents had an OR 2.64 [95% CI 1.36-5.11], p=0.008.

Among clinical manifestations, asthma, ENT involvement, and peripheral neuropathy were significantly associated to all the risk factors. Regarding to asthma, silica exposure showed an OR 2.55 [95% CI 1.37-4.74], p=0.003, farming exposure an OR 2.61 [95% CI 1.61-4.20], p=0.001, the exposure to organic solvents an OR 3.15 [95% CI 1.85-5.38], p<0.0001. Regarding to ENT involvement, silica exposure had an OR 2.46 [95% CI 1.29-4.65], p=0.008, farming exposure had an OR 2.26 [95% CI 1.38-3.72], p=0.001, the exposure to organic solvents had an OR 3.18 [95% CI 1.84-5.50], p<0.0001. Finally, peripheral neuropathy was associated with the exposure to silica (OR 2.60 [95% CI 1.34-5.07], p=0.008, to farming (OR 2.43 [95% CI 1.44-4.11], p=0.001, and to solvents (OR 2.89 [95% CI 1.61-5.19], p=0.0008.

DISCUSSION

The pathogenesis of EGPA is still poorly understood and matter of debate. Certainly, the disease susceptibility is related to many immunogenetic factors. However, little is known about aetiological or triggering agents. Studies analyzing the role of drugs in EGPA have shown conflicting results [17-21]. Studies performed to investigate the role of environmental or occupational risk factors in AAV were underpowered and limited by the inclusion of control subjects with nephrological or rheumatological diseases [16]. In addition, these studies are usually aimed to analyze only a specific risk factor (i.e. silica or farming) and do not include EGPA patients or only a small number of them [16, 89, 90, 93].

In our study, we showed that exposure to silica, farming and organic solvents may be associated with an increased risk of EGPA. Silica exposure has been associated with a number of autoimmune diseases including other AAV, rheumatoid arthritis [101] and systemic lupus erythematosus [102]. The mechanisms through which silica results in autoimmunity are not fully understood, but are thought to include its potential action as a non-specific immune adjuvant for activation of responder T cells and an early apoptosis of regulatory T cells [103]. We know that EGPA pathogenesis is not related only to the activation of the Th1 pathway. However, silica particles are also considered to induce apoptosis of monocytes, macrophages, and possibly neutrophils leading to the Th2 pathway activation. Surface expression of MPO during apoptosis of neutrophils in the absence of priming has been shown. The ANCA may bind to the antigen on apoptotic cells, resulting in an amplified release of cytokines, oxygen radicals, and lysosomal enzymes operative in vasculitis [95]. In addition, we confirm that the risk of developing the disease correlates with the duration of silica exposure, as already reported by previous studies in AAV [16]. This may reflect the role of silica in the pathogenesis: it may act not only as trigger of disease but also as autoimmune adjuvant. However,

our questionnaire was not designed to assess the levels or the timing of exposure (recent versus past exposures) and more studies are required to clarify this issue.

Our study reports for the first time an association between EGPA and farming. Two previous studies investigating farming exposure in AAV included EGPA and did not found an association with this disease [16, 93]. However, they included respectively only 16 and 22 EGPA patients and the lack of association could be related to the low number of patients. We were not able to distinguish between specific farming exposures (livestock or crops). However, we did not find an association between EGPA and pesticides. Thus, one explanation about the aetiological role of farming in EGPA is that infectious agents affecting animals may play a role in the onset of the disease. Previous studies, indeed, reported that Paramyxovirus and Pseudorabies virus affecting pigs may cause some vasculitis manifestations in farmers [104, 105]. Alternatively, other factors in the care of livestock may be important, including feeds, antibiotics and disinfectants.

We also found a multiplicative effect on the risk when there was a coexposure to silica and farming compared with single exposures. Such an effect appears biologically plausible because, if farming could act as a trigger of the disease, on the other side silica exposure may sustain chronic autoimmune responses. Certainly, this hypothesis deserves further investigation.

Lane et al. already reported an association between a prolonged exposure to solvents and AAV. However, in their study, solvents did not appear associated with EGPA alone. Other authors reported the association between solvents and renal vasculitis [91]. We found a significative association between solvents and EGPA but this association was not influenced by the exposure duration. In addition, we failed to find an association between this risk factor and kidney involvement. However, it must be considered that renal manifestations are rare in EGPA and a study including a larger number of patients should be performed in order to investigate the associations with the different disease manifestations.

Finally, we identified smoke exposure as a protective factor in the development of EGPA. Two previous case-control studies investigated the association between smoking habits and AAV. They failed to found an association, probably due to the control groups, which included patients with other immunological or pulmonary diseases and not healthy controls. Two descriptive and retrospective studies analyzed smoking habits in patients with AAV. Both papers reported a very low incidence of current and former smokers than that reported in general population by the national statistical Institute [97, 106]. We found that smoking exposure is less frequent in patients than in controls not only at the time of diagnosis but also before the onset of the prodromes of the disease, which usually are represented by asthma and nasal polyposis and may influence the smoking status of the patients. However, our findings are not surprising. It has already been reported that nicotine reduces Fas/Fas ligand signaling in lymphocytes, leading to the impairing of T cell signaling [107]. In addition, carbon monoxide may inhibit apoptosis of vascular smooth muscle cells and inhibit thrombosis [108]. We can speculate that, acting especially in the upper airways, carbon monoxide and nicotine may inhibit immune responses involved in the development of EGPA, usually stimulate by inhaled agents, such as silica or farming.

Our study has both strengths and limitations. Strengths include the quite large size of the patient cohort and the prospective enrolment, the adequate match with controls and the 1:3 ratio between cases and controls. In addition, this is the only study in which many occupational and environmental agents and individual conditions are investigated as risk factors for this disease. The limitations of our study include instead the fact that it is a questionnaire-based study, and participants may have had difficulty to ascertain or quantify exposure to specific agents. In addition, the exclusion of 12 patients because of death or loss to follow up may have introduced a bias in terms of variables investigated. Finally, the findings regarding clinical manifestations are very difficult to interpret and

certainly more studies including larger cohorts are necessary in order to confirm the associations between single organ involvement and particular risk factors.

In conclusion, our study has demonstrated, for the first time, a plausible, significant association between EGPA and exposure to silica, farming and organic solvents, supporting the results of previous studies which identified these risk factors in AAV. There is a relationship between the duration of silica exposure and the risk of disease development and there is a multiplicative effect of the co-exposure to silica and farming on the risk of disease development. Tobacco smoking appears to be a protective factor against EGPA development. We believe that the associations we found are sufficiently strong to warrant further studies aimed to investigated the biological effects of risk factors on disease pathogenesis.

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FIGURES AND TABLES

Figure 1. Study flow diagram: referral patterns and selection of patients with EGPA for enrollment.

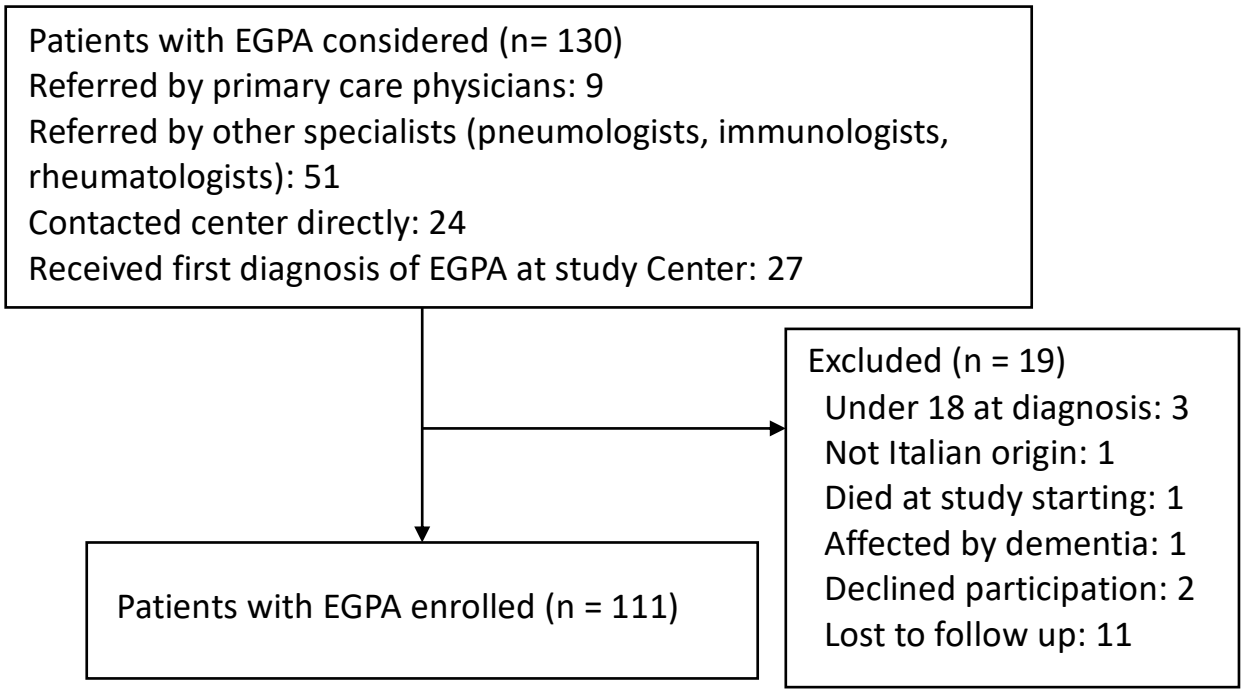

Figure 2. Study flow diagram: selection and enrollment of control participants**.**

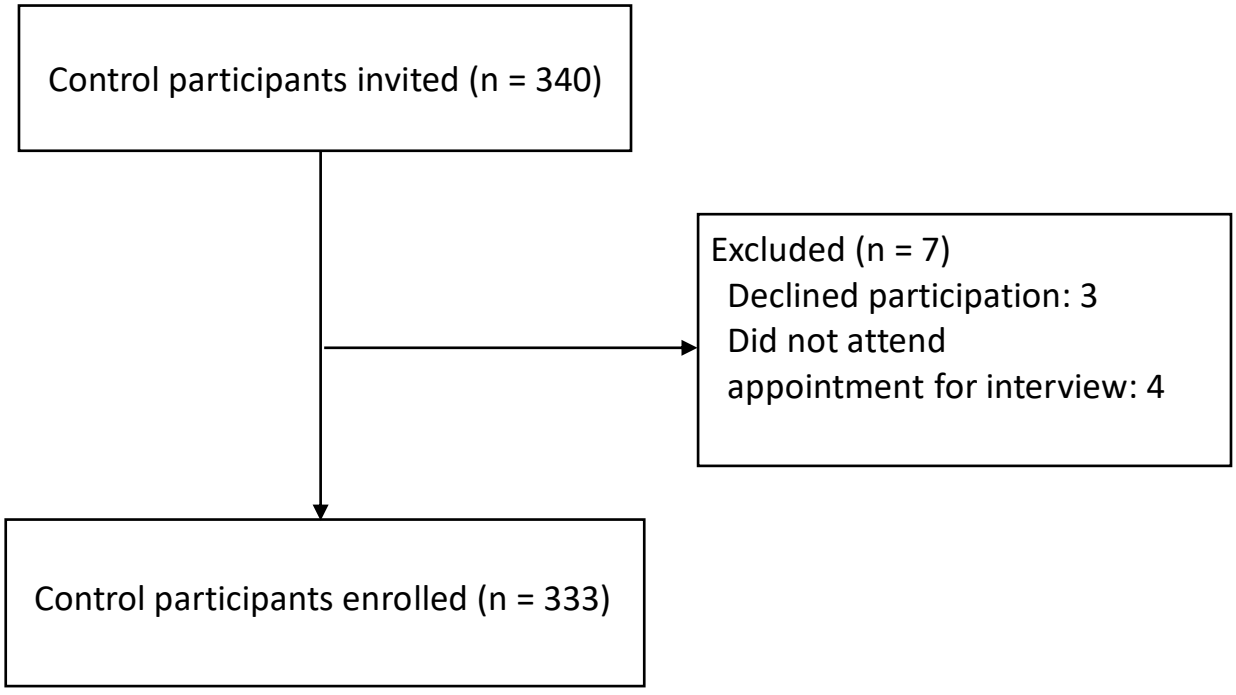

Table 1. Demographic and clinical characteristics of the study participants.

	Cases (N = 111)
Age at diagnosis - median (IQR), years	53 (44.5-61)
Sex – no. (%)	
Male	53 (48)
Female	58 (52)
Geographic origin – no. (%)	
Northern Italy	47 (42)
Central-Southern Italy	64 (58)
Disease manifestations – no. (%)	
Cardiac	22 (19.8)
Cutaneous	35 (31.5)
Gastrointestinal	16 (14.4)
Asthma	106 (95.5)
Lower respiratory tract	62 (55.8)
Peripheral neuropathy	78 (70.3)
Central nervous system	5 (4.5)
Ear-Nose-Throat	92 (82.9)
Renal	12 (10.8)
Disease activity	
BVAS - median (IQR)	13 (7–17)
Five-Factor score (FFS) – no (%)	
FFS = 0	50 (45.0)
FFS ≥ 1	61 (55.0)
ANCA-positive – no (%)	
By immunofluorescence	
c-ANCA	8 (7.2)
p-ANCA	50 (45.0)
By ELISA	
Anti-PR3	5 (4.5)
Anti-MPO	46 (41.4)

BVAS = Birmingham vasculitis score; ANCA = antineutrophil cytoplasmic antibodies; PR = proteinase-3; MPO = myeloperoxidase

Table 2. Prevalence of co-morbidities and non-occupational risk factors in cases and controls

Risk factor	Cases N = 111	Controls N = 333	Crude OR (95% CI)	*P*-value
BMI (kg/m^2) - median (IQR)	24 (22-26)	25 (23-28)	0.90 (0.84-0.96)	0.001
Obese (BMI ≥ 30 kg/m^2) - n (%)	7 (6)	38 (11)	0.52 (0.23-1.21)	0.128
Comorbidities				
Hypertension - n (%)	28 (25)	133 (40)	0.66 (0.41-1.07)	0.089
Diabetes - n (%)	5 (5)	20 (6)	0.74 (0.27-2.02)	0.554
Ischemic heart disease - n (%)	3 (3)	40 (12)	0.20 (0.06-0.67)	0.009
Cerebrovascular disease - n (%)	1 (1)	6 (2)	0.50 (0.06-4.16)	0.518
Cancer - n (%)	12 (11)	13 (4)	2.76 (1.24-6.17)	0.013
Smoke exposure at diagnosis				
Nonsmokers - n (%)	73 (66)	154 (46)	-	-
Former smokers - n (%)	36 (32)	83 (25)	0.92 (0.57-1.48)	0.717
Current smokers - n (%)	2 (2)	96 (29)	0.04 (0.01-0.18)	<0.001
Former/Current smokers - n (%)	38 (34)	179 (54)	0.49 (0.29-0.70)	<0.001
Pack/year index - median (IQR)	10 (5-20)	18 (9-30)	-	0.014
Smoking exposure at asthma onset (cases)	**Cases N = 111**	**Controls N = 222**		
Age – median (IQR)	43 (36-52)	45 (38-52)	-	0.55
Nonsmokers – n (%)	75 (67)	93 (42)	-	-
Former/Current smokers – n (%)	36 (32)	129 (58)	0.34 (0.21-0.56)	<0.0001

OR = odds ratio; BMI = body mass index

Table 3. Prevalence of occupational risk factors in cases and controls (univariate analysis).

Risk factor	Cases N = 111, n (%)	Controls N = 333, n (%)	Crude OR (95% CI)	*P*-value
Chemicals	32 (29)	60 (18)	1.84 (1.12-3.03)	0.016
Metallic elements	13 (12)	42 (13)	0.92 (0.47-1.78)	0.803
Pesticides	11 (10)	28 (8)	1.20 (0.58-2.49)	0.629
Silica	24 (22)	30 (9)	2.79 (1.55-5.01)	0.001
Organic solvents	35 (32)	42 (13)	3.19 (1.91-5.34)	<0.001
Asbestos exposure				
Non-exposed	56 (50)	227 (68)	-	-
Occupational (documented)	3 (3)	13 (4)	0.94 (0.26-3.40)	0.919
Occupational (possible/probable)	17 (15)	29 (9)	2.38 (1.22-4.63)	0.011
Occupational (all)	20 (18)	42 (13)	1.93 (1.05-3.54)	0.034
Extra-occupational	35 (32)	64 (19)	2.22 (1.34-3.67)	0.002
Textiles fibers	21 (19)	38 (11)	1.81 (0.99-3.24)	0.062
Farming	47 (42)	71 (21)	2.71 (1.71-4.29)	<0.001

OR = odds ratio

Figure 3. Odds ratios for EGPA associated with duration of silica and chemical exposures.

Top. Compared to subjects not exposed to silica, those below the median duration of silica exposure had an OR lower than those above the median duration of the exposure. **Bottom.** The analysis of the duration of chemical exposure revealed an association with a long-term exposure while no association was demonstrated between EGPA and chemical exposure lasting less than the median duration of the exposure.

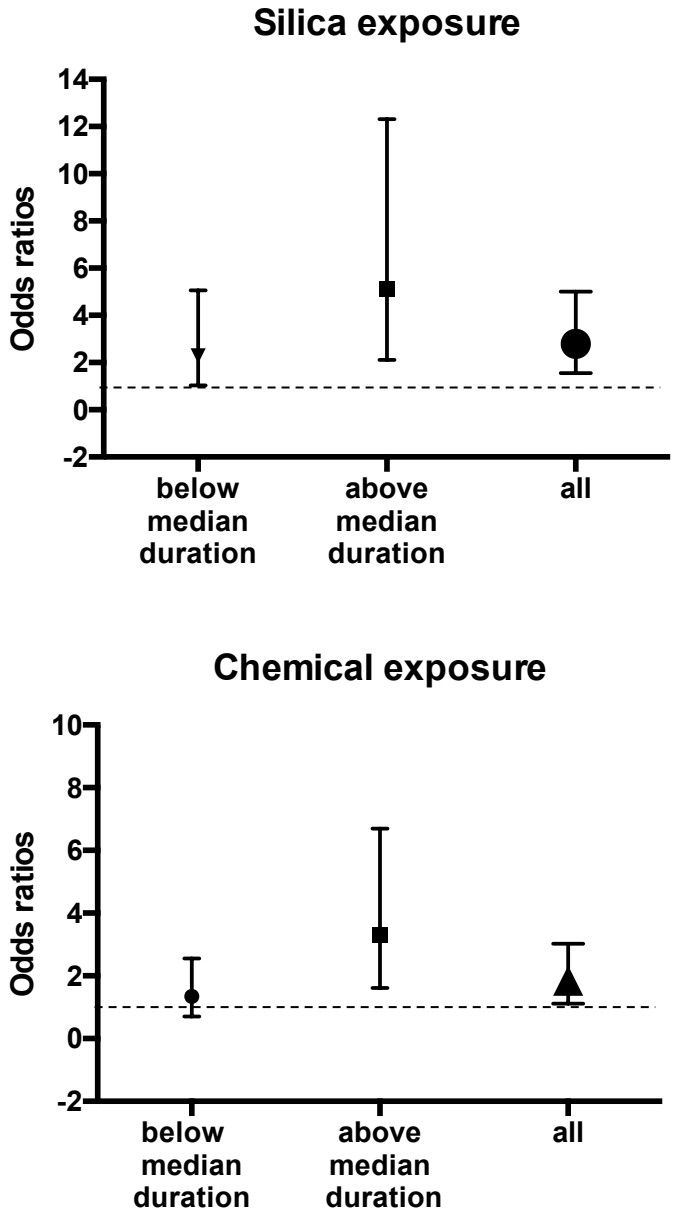

Table 4. Association between exposure to risk factors and EGPA (Conditional Logistic Regression Model).

Variable	OR (95% CI)	*P*-value
Smoke exposure (Former/Current smokers)	0.39 (0.22-0.69)	0.001
Silica	2.26 (1.10-4.62)	0.026
Organic solvents	2.20 (1.14-4.25)	0.018
Farming	2.10 (1.19-3.73)	0.011

OR = odds ratio

Figure 4. Coexposure to farming and silica. The coexposure to farming and silica led to an OR higher than the addition of the single OR, suggesting a multiplicative (rather than just an additive) effect between these two variables.

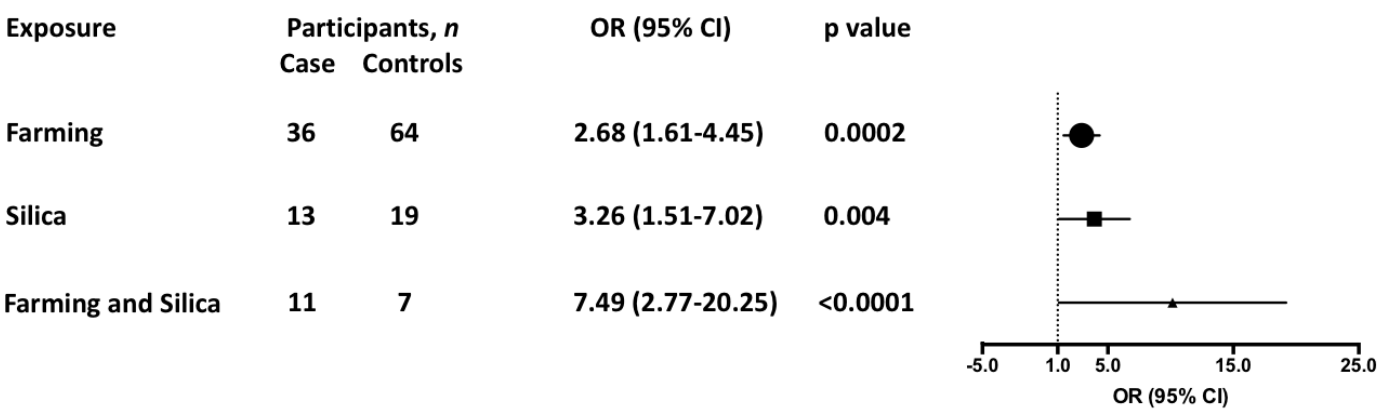

Table 5. Associations with risk factors for EGPA, ANCA positivity and disease manifestations.

EGPA subtypes	Silica OR (95% CI)	*P*-value	Farming OR (95% CI)	*P*-value	Organic solvents OR (95% CI)	*P*-value
ANCA positive	3.85 (1.93-7.65)	0.0002	2.60 (1.45-4.67)	0.002	2.64 (1.36-5.11)	0.008
Asthma	2.55 (1.37-4.74)	0.003	2.61 (1.61-4.20)	0.001	3.15 (1.85-5.38)	<0.0001
ENT involvement	2.46 (1.29-4.65)	0.008	2.26 (1.38-3.72)	0.001	3.18 (1.84-5.50)	<0.0001
Pulmonary involvement	3.51 (1.77-6.95)	0.0007	2.66 (1.50-4.70)	0.001	1.80 (0.88-3.68)	0.103
Gastrointestinal involvement	2.33 (0.63-8.64)	0.184	1.23 (0.38-3.93)	0.756	1.15 (0.13-9.83)	1.000
Cardiac involvement	4.71 (1.78-12.47)	0.004	1.08 (0.38-3.04)	0.794	2.04 (0.71-5.81)	0.190
Peripheral neuropathy	2.60 (1.34-5.07)	0.008	2.43 (1.44-4.11)	0.001	2.89 (1.61-5.19)	0.0008
Skin involvement	2.52 (1.02-6.27)	0.068	4.38 (2.14-8.96)	<0.0001	2.39 (1.05-5.47)	0.041
Kidney involvement	3.36 (0.86-13.11)	0.096	2.63 (0.81-8.55)	0.146	3.46 (0.99-12.01)	0.061

OR = odd ratio; ENT = ear, nose, throat.