The role of metals in autoimmune vasculitis: epidemiological and pathogenic study

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Abstract

Background: A possible relationship between Silica (Si) exposure and antineutrophil cytoplasm antibodies (ANCA)-associated vasculitis has been reported. Furthermore, tuberculosis (TBC) has been frequently described in patients with silicosis, and TBC infection shares with ANCA-associated vasculitis the formation of granulomas. Therefore, an intriguing network including Silica, Vasculitis, TBC and ANCA might be hypothesized. The aim of this work was to further investigate these correlations using both epidemiological and pathogenic approaches. *Methods*: *Study I — epidemiological study.* A case–control study to compare the occupational histories of 31 cases of biopsy proven vasculitis (18 pauci-immune crescentic glomerulonephritis, 9 microscopic polyangitis, 4 Wegener's granulomatosis) with those of 58 age, sex and residence-matched controls (affected by other kidney diseases), was performed. Occupational Health physicians designed an appropriate questionnaire in order to evaluate a wide spread of exposures and calculate their entity by the product of Intensity × Frequency × Duration. *Study II — tuberculosis association.* A case–control study to evaluate the frequency of a previous history of tuberculosis (TBC) in 45 patients with vasculitis and 45 controls were performed. *Study III — ANCA positivity.* A case–control study to evaluate the presence of ANCA was performed by testing blood samples of 64 people with previous professional exposure and 65 sex/age matched patients hospitalized in a General Medicine Unit. Furthermore, the same evaluation was made in a pilot study in 16 patients with ongoing or previous TBC. *Study IV — experimental study.* The oxygen free radicals

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(OFR) and IL-12 production (both involved in the pathogenesis of vasculitis) from human phagocytic cells stimulated with an amorphous (diatomaceous earth) and a crystalline (quartz) form of Si at the doses of 10 and 100 $\mu g \, ml^{-1}$ was evaluated. *Results*: Study I — a positive history of exposure to Si resulted in significantly more present in cases (14/31 = 45%) than in controls (14/58 = 24%, $P = 0.04$, OR = 2.4) and no other significant exposure association was found (including asbestos, mineral oil, formaldehyde, diesel and welding fumes, grain and wood dust, leather, solvents, fungicides, bitumen, lead and paint). Study II — past TBC infection was significantly more present in patients with vasculitis (12/45 = 26%) than in controls (4/45 = 8%, $P < 0.05$). Study III — ANCA was present in 2/64 exposed people (vs. 0/65 controls, P = NS) and 0/16 patients with TBC. Study IV — both amorphous and crystalline Si forms represented a stimulus for OFR and IL-12 production, but quartz resulted as a greater inductor. *Conclusions*: We conclude that Si exposure might be a risk factor for ANCA-associated vasculitis, possibly enhancing endothelial damage by phagocyte generation of oxygen free radicals and Th1 differentiation by an excessive IL-12 phagocyte production. Frequency of TBC was significantly higher in vasculitis patients. ANCA was not frequent in the preliminary examination of people with previous professional exposure or patients with TBC, but the number of samples evaluated is too small to allow conclusions. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

There is clinical and experimental evidence that silica exposure affects the immune response. Many examples from literature show an association between silica exposure and clinical and serological manifestation of autoimmune diseases such as systemic sclerosis, rheumatoid arthritis, Chron's disease (Koeger, 1994) systemic lupus erythematosus (SLE), dermatomyosis, polymiositis, overlap syndrome (Sanchez-Roman et al., 1993) and renal vasculitis (Gregorini et al., 1997; Cohen Tervaert et al., 1998).

Among serological markers of autoimmune disease, rheumatoid factor and antinuclear antibodies have been found in 26–44% of silicotic patients (Wilke et al., 1996). In addition, a few studies reported high levels of anti-neutrophil cytoplasm antibodies (ANCA) against myeloperoxidase (MPO) in some individuals with previous exposure to silica (Wichmann et al., 1996; Gregorini et al., 1997). Cytoplasmic-ANCA (C-ANCA) and perinuclear-ANCA (P-ANCA) are autoantibodies against proteinase-3 and myeloperoxidase (MPO), respectively, which are two proteins contained in neutrophils granules (Jennette and Falk, 1990; Savige et al., 1990; Kallenberg et al., 1992). These autoantibodies take an important role in the diagnosis, prognosis and maybe pathogenesis of renal vasculitis (Lesavre, 1996). The ANCA are closely associated with three major categories of

small-vessel vasculitis: Wegener's granulomatosis (WG) (Hoffman, 1995), microscopic polyangiotis and extracapillary/necrotizing glomerulonephritis (GN) without immune deposits as seen with the immunofluorescent techniques. This last form is considered a type of vasculitis confined to renal capillary bed, as assumed from the absence of signs of overt involvement of other organs or apparatus (Jennette and Falk, 1990; Jennette et al., 1994; Jennette and Falk, 1997; Ferrario et al., 1994; Van der Wood and Ferrario, 1999). However, pulmonary involvement, which is the rule in Wegener's granulomatosis (over 90% of patients), is a common feature in microscopic polyangitis and may occur also in patients with extracapillary pauci-immune GN, eventually leading to a picture which is called by some authors 'the reno-pulmonary syndrome' (Brouwer et al., 1994; Griffith Megan et al., 1996).

What is the possible link between silica and ANCA/silica and vasculitis? Firstly, data from the literature have already demonstrated a production of oxygen-free radicals (OFR) from polymorphonuclear (PMN) leukocytes stimulated with crystalline silica dust: OFR are mediators of flogosis and this mechanism could trigger a pathway eventually leading to endothelial damage such as that observed in vasculitis (Gusev et al., 1993). Secondly, the interaction between silica with cells might induce the exposure of MPO on the cell

membrane and its release by PMN and mono-cytes. It would be possible to hypothesize that silica inhalation promotes an autoimmune reaction by the release of an antigen (MPO) capable to be recognized by an autoantibody (ANCA). Thirdly, a role for silica might be suggested in inducing cellular production of other flogistic cy-tokines (Bolton et al., 1981; Bonnin et al., 1987; Dracon et al., 1990; Neyer et al., 1994; Fubini, 1998; Fubini, in press). Recently, IL-12-dependent autoimmune responses have been demonstrated to exacerbate or cause autoimmune diseases. In particular, Th1 response was supposed to be associated with the granulomatous inflammatory lesion in Wegener's granulomatosis.

As for the association between TBC infection and vasculitides, this has been reported in anne-doctical studies (Mishima et al., 1994; Han et al., 1995; Houman et al., 1996; Hernandez-Pando et al., 1994; Aggarwal et al., 1996). These observations are intriguing because (1) TBC and vasculitis may share some common pathway of cellular reaction eventually leading to granulomatous formation, and (2) TBC has been reported with high frequency also in patients with previous silica exposure, but no systematical assessment of the data was realized and there are no correlations with ANCA-vasculitides.

In this study, the authors planned to further investigate this network of relationships, by evaluating:

- the correlation between silica and vasculitides using both an epidemiological and a pathogenetic approach;
- the incidence of previous history of tuberculosis (TBC) in patients with vasculitis; and
- the frequency of ANCA positivity in subjects with professional exposure to silica or TBC infection.

As for the pathogenesis, in the present study the relationships between Si and functional responses of cell involved in autoimmunity (PMN and monocytes) has been examined, with particular attention to the production of OFR and PMN production of IL-12. OFR production will be used

as a parameter of cell activation to which compare the other cellular events in the study. In addition, the ability of different types of silica dusts in inducing cell activation and IL-12 production by PMN and monocytes has been tested.

2. Patients and methods

2.1. Clinical studies

2.1.1. Study I

A case-control study was performed to compare the occupational history of patients affected by vasculitis with those of patients with other nephropathies. Among sixty-one cases of renal-related vasculitis who underwent renal biopsy at the Department for Nephrology of the University of Turin between 1976 and 1998 (35 men and 26 women of mean ages 58 ± 12, range $31-79$ years at the time of the diagnosis) it was possible to carry out the evaluation of professional exposure on 31 cases, because these were still alive or traceable (the study must be carried out on patients who are alive in order to guarantee the collection of occupational history records which is impossible to obtain just from the patients' records). According to renal histology, the patients were subdivided in agreement with the Chapell Hill (1994) criteria, as follows:

- Sixteen extracapillary glomerulonephritis (renal vasculitis);
- nine microscopic polyangitis; and
- six Wegener's granulomatosis.

Among 117 controls, 58 were examined — 31 men and 27 women. They were all nephropatic patients, not suffering from vasculitis. Controls were matched for sex, age (within 4 years) and region of residence, subdividing the territory of our Piemonte Region into five areas: 1, the city of Turin; 2, the first residential outskirt; 3, the second residential outskirt; 4, the Province of Torino; 5, other Provinces. In the case of patients coming from other Italian regions, controls coming from the same or neighboring region were selected.

Renal diseases in the 'Control' group were subdivided as follows:

- 16 primitive glomerulonephritis (GN);
- 5 secondary GN;
- 6 vascular nephropaty;
- 10 diabetes nephropaty;
- 3 paraproteinemiae-related renal disease; and
- 18 others (tubulo-interstitial, polycistic, etc.).

2.1.1.1. Occupational exposure survey. A structured questionnaire, prepared together with industrial hygienists, was administered to each subject. This was filled following current epidemiological methodologies (Porro et al., 1992; Nuyts et al., 1995a,b).

Mainly silica and asbestos exposures were taken into consideration, but also other substances were defined, when activity exposure to other substances was reported in the same working environment. In particular, exposure to the following other substances was evaluated: lead, copper, cadmium, chromium, aluminum, tin, mercury, welding fumes, diesel and petrol exhaust fumes, mineral/lubricant oils, grain and cereal dust, wood, leather and hide dust, formaldehyde, bitumen, tar, pitch, all solvents and hydrocarbons. With multiple exposures, each substance has been evaluated.

For each job held longer than 6 months the following features were reported:

1. job status;
2. company name;
3. sector;
4. years worked (date of beginning and end) for each job held;
5. detailed description of task performed; and
6. working environment.

Structured questionnaires have been created for points (5) and (6), i.e. regarding the following sectors: engineering, welding, building, bodywork, galvanization, textile, mining industries, asbestos industry (including clutch, gasket and sealing materials) and asbestos–cement industry. These enabled the interviewed person to precisely indicate his/her working environment, the machinery and materials used, the substances he/she has been exposed to.

The type and entity of the exposure was blindly scored by an industrial hygienist with the following parameters:

- probability;
- degree; and
- frequency.

The probability (P) of exposures is a 'characteristic' of the technology employed in the sector or division examined and express the variance of productive processes performed in a specific combination of period and area. It is coded with values from 0 (no probability) to 4 (maximum probability). If the probability is higher than none, degree (I) and frequency (F) are measured.

The degree of exposure is classified according to five levels:

- 0 = none (as for general population);
- 1 = uncertain or low exposure;
- 2 = moderately superior exposure to that of the general population;
- 3 = moderate intensity exposure; and
- 4 = high intensity exposure.

Also, frequency of exposure is classified according to five levels:

- 0 = none or merely occasional;
- 1 = > 0 ≤ half a day per week;
- 2 = > half a day per week ≤ 1 day per week;
- 3 = > 1 day per week ≤ 3 days per week; and
- 4 = > 3 days per week.

The total score of each exposure in each job (cumulative exposure) was calculated as the product of degree, frequency and duration of the job ($I \times F \times D$). When different types of jobs were exposed to the same substance, the total score would derive from the sum of cumulative exposure of that substance of each job. From the above score three degrees of exposure were defined: none, when cumulative exposure equaled 0, low or high depending on whether the cumulative

score was higher than the 75th percentile of the distribution scores of the exposed cases and controls, and low exposure if any score was lower than the 75th percentile.

2.1.2. Study II

For the analysis of past TBC, a case-control study was performed. Among the 61 patients with vasculitis 45 were examined, because sufficient data were not available for the others. The patients (27 males, 18 females, mean age 58 ± 12 years) were affected by biopsy-proven renal vasculitis: 21 pauci-immunis extracapillary GN; 13 micropoliangioitis; and 11 WG. A comparison was made with 45 sex, age and residence matched controls affected by other types of nephropaty. For a previous tuberculosis infection to be diagnosed, the following criteria were employed:

- previous overt clinical diagnosis (patient report and/or patient records); and
- radiological diagnosis (only if an explicit radiologist statement of TBC sequela was present).

2.1.3. Study III

Evaluation of ANCA-MPO and ANCA-Pr3 positivity prevalence (ELISA) was performed in 64 subjects with previous professional exposure. Forty-four subjects (43 men, 1 woman, mean age 67 ± 10 years) had been exposed to silica dust; 33 of them suffered from overt silicosis, including 4 with TBC superimposed on pulmonary silicosis. Twenty subjects (11 men, 9 women, mean age 60 ± 9 years) had been exposed to asbestos. Autoimmune and/or renal diseases were not recorded in past or present records of all 64 subjects.

The group of controls constituted of 65 patients (45 men, 20 women, mean age 76 ± 10 years) who were hospitalized in the Geriatric Medicine Unit of our Hospital at the time of the study. Their clinical history was free from previous and present autoimmune diseases and occupational risk exposure to environmental pollutants was unknown. A further pilot study was performed by looking for ANCA-positivity in 16 patients (9 males, 7 females, mean age 54 ± 17 years) with

active ($n = 7$: 6 with pulmonary, 1 with lymphonodal involvement), past ($n = 6$: 4 pulmonary, 2 extrapulmonary involvement) and relapsing ($n = 3$: 2 pulmonary, 1 retroperitoneal) TBC infection. Among these patients, previous occupational exposure to silica dust and/or asbestos was documented in four cases.

2.2. Experimental study

2.2.1. Study IV

Relationships between silica and functional responses of cell involved in autoimmunity (leukocytes and monocytes) were examined, with particular attention to: (1) production of OFR; and (2) production of IL-12. Three types of silica were used: (a) Min-U-sil — crystalline silica constituted by single particles of diameter < 5 μm with irregular and indented borders (5 m^2 g^{-1} surface); (b) diatomaceous earth — natural amorphous silica constituted by irregular particles derived from algae with different diameters (50 m^2 g^{-1} surface); and (c) aerosil — synthesized amorphous silica constituted by very small single particles of diameter of approximately 50 nm with regular and smooth borders (5.2 m^2 g^{-1} surface). Silica dusts were suspended in saline solution at final concentrations of 10, 100, 1000 and 5000 μg ml^{-1}.

2.2.1.1. Preparation of human PMN. Human PMN were isolated from the venous blood of healthy donors by sequential centrifugation and gelatin sedimentation (2.5% gelatin in PBS, pH 7.2, for 30 min at 37°C), as previously described (Camussi et al., 1988). Contaminating erythrocytes were removed by hypotonic lysis and the cells resuspended in RPMI 1640 medium at the final concentration of 3×10^6 ml^{-1}. The percentage of PMN in the cell preparation used in this study is 95–97%.

2.2.1.2. Preparation of human monocytes. Monocytes were isolated from PBMC by adhesion to plastic dishes, as described by Valone and Epstein (1988). Non-adherent cells were removed by vigorous washing and 1 ml RPMI 1640 (GIBCO, Paisley, Scotland, UK) containing 0.25% BSA was

then added to each well. Adherent cells in representative wells were removed by scraping with a rubber policeman and counted. The number of cells recovered per well was $2.5 + 0.2 \times 10^6$ (mean + 1 S.D. for 10 consecutive studies). Adherent cells was > 90% monocytes, as detected by non-specific esterase staining and immunofluorescence positivity with the anti-CD14 LeuM3 mAb. Less than 1 platelet/10 monocytes was detected. Monocytes viability was assessed by Trypan blue dye exclusion test. Monocytes were also separated on the basis of CD14 expression by magnetic cell sorting, using the MACS system (Miltenyi Biotec). Briefly, PBMC in MACS buffer (10^7 cells/80 µl) were labeled with the CD14 mAb conjugated with MACS super-paramagnetic microbeads (Miltenyi Biotec) for 20 min at a concentration of 20 µl 10^{-7} cells at 6−12°C. After washing twice in MACS buffer, cells were separated on a magnetic stainless steel wool column (Miltenyi Biotec), according to the manufacturer's recommendations. CD14-positive cells, attached to the magnetized matrix, were obtained after removal of the column from the magnet and were flushed with MACS buffer into another tube. Cytometric analysis of the collected cells was performed.

2.2.1.3. OFR production. Production of superoxide anion (O_2^- assay) has been measured as the superoxide dismutase-inhibitable reduction of ferricytochrome C (Rovin et al., 1990) 100. Cells (2.5×10^6 cells) were incubated at 37°C in Tyrode's buffer (2.6 mM KCl, 1 mM $MgCl_2$, 137 mM NaCl, 6 mM $CaCl_2$, 0.1% glucose, 1 mM Tris, pH 7.4) containing 80 µM cytochrome C with or without SOD (50 U ml^{-1}) and appropriately stimulated with different doses and types of silica dust. As a control, TiO_2 was used. Basal O_2^- production was assessed in the absence of stimulating factors. Supernatants were removed at specified times and centrifuged, and the absorbance measured in a spectrophotometer at 550 nm. The extinction coefficient of ferricytochrome C at 550 nm was taken as 2.1×10^4 M^{-1} cm^{-1} (Hedenborg and Klockars, 1989; Vallyathan et al., 1988). Protein content of PMN was measured according to the Lowry technique. O_2^- produc-

tion was expressed as nanomoles of cytochrome C reduced per milligram of protein.

2.2.1.4. Cytokine detection. The quantitative determination of IL-12 in the supernatant of PMN and monocytes stimulated for 12, 24 and 48 h with different types and doses of silica dust was performed by ELISA using specific kits (Genzyme). Lypopolysaccharides stimulation was used as a positive control.

2.3. Biochemical studies

Anti MPO (P-ANCA) and anti Pr3 (C-ANCA) were evaluated in serum samples of cases and controls by ELISA techniques (Medic).

2.4. Statistical analysis

Data are presented as mean ± S.D. Comparison are made by employing Student's *t*-test and χ^2 analysis. A *P* value < 0.05 is considered statistically significant.

3. Results

3.1. Epidemiological study

3.1.1. Study I

As for a generic risk of professional exposure, there was not a significant difference between the two populations: 22/31 patients affected by vasculitis (70%) and 36/58 (62%, *P* = 0.54) controls reported a working history with exposure to environmental pollutants (Table 1). An exposure to different pollutants was frequent, mainly among the cases (patients with vasculitis): in 16 out of 22 exposed cases and in 18 out of 36 exposed controls (patients with other type of renal diseases) an exposure to ≥ 3 pollutants during life was recorded.

In both groups, women represented only approximately 30%. Fifty-seven percent of women affected by vasculitis and 44% of women belonging to the control group resulted free from every working exposure here investigated. On the contrary, 94% of men affected by vasculitis and 77%

Table 1
Occupational exposure (every) in cases and controls: distribution

Subjects	Occupational exposure	
	At least 1 risk n (%)	No risk n (%)
Cases (renal vasculitis)		
Men ($n = 17$)	16 (94)	1 (6)
Women ($n = 14$)	6 (43)	8 (57)
Total ($n = 31$)	22 (71)	9 (29)
Controls (other renal diseases)		
Men ($n = 31$)	24 (77)	7 (22)
Women ($n = 27$)	12 (44)	15 (55)
Total ($n = 58$)	36 (62)	22 (38)

of men affected by other types of nephropaty showed one or more pollutants contacts during their working history. In both the populations the women appeared as significantly less victims to professional exposure (6 women among 22 people exposed = 27% vs. 8/9 = 89% in non-exposed cases, $P = 0.0016$; 12/36 = 33% in exposed vs. 15/22 = 69% in non-exposed controls, $P = 0.02$).

Among different pollutants, silica, asbestos, oils and solvents were most common in both groups of patients (Fig. 1). Exposure to silica was significantly more frequent in patients with vasculitis
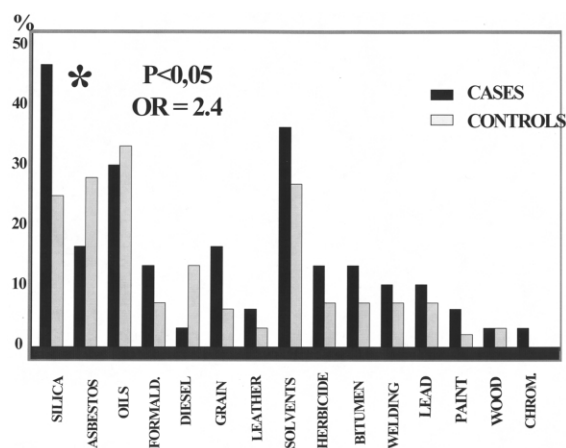

Fig. 1. Frequency of different types of occupational exposure in patients with vasculitis (cases) and with other renal diseases (controls).

Table 2
Silica exposure in 31 cases: distribution

Types of vasculitis	N	Exposed	%
Wegener	6	3	50
Micropoliangioitis	9	5	55
Rapidly progressive GN	16	6	37
Total	31	14	45

(14/31 = 45%) than in controls (14/58 = 24%, $P = 0.04$, OR = 2.4) and no other significant differences were found in frequency of exposure. Among patients with vasculitis, previous silica exposure was recognized in a moiety of cases with Wegener and micropoliangioitis and in 37% of cases with vasculitis limited to renal glomeruli ($P = 0.4$) (Table 2). Among patients with other renal diseases, previous silica exposure was recognized mainly in cases of primary glomerular diseases and vascular diseases, but the differences did not reach the level of statistical significance ($P = 0.1$) (Table 3). Silica exposure has been documented in five farmers, four blue collars, two construction workers, two miners and in one sandblaster of the group of patients with vasculitis and in five farmers, four blue collars and in six construction workers of the group of patients with other renal diseases. Overt clinical silicosis has been diagnosed only in three patients with vasculitis, but no differences were found between the intensity of exposure to silica in the two groups [$(P \times I \times F) < 80$, corresponding to a low degree exposure in both].

In patients with renal vasculitis, the time of exposure to silica lasted from 4 to 45 years (in farmers) with a mean value of 14 years. The lag time from silica exposure to the clinical onset of

Table 3
Silica exposure in 58 controls: distribution

Types of vasculitis	N	Exposed	%
Primary GN	16	7	43
Secondary GN	5	1	20
Vascular renal disease	6	3	50
Diabetes	10	1	10
Others	21	2	9.5
Total	58	14	24

renal vasculitis ranged from 6 to 39 years (mean 25 years) except for farmers, in whom renal diseases developed when occupational exposure was still ongoing.

A last analysis was performed by considering the degree of renal damage. Nine of 31 patients (29%) with renal vasculitis eventually developed end-stage renal failure needing chronic dialysis: six of them (70%) had been exposed to silica with a degree of exposure at the top of the ranges observed in the overall population analyzed in this study.

3.1.2. Study II

The results of the TBC analysis showed a statistically significant difference ($P < 0.05$) in the presence of past TBC infection in patients affected by vasculitis (12/45) compared to controls (4/45). Distribution of TBC infection was as follows: 4/11 (36%) Wegener, 4/13 (31%) micropoliangioitis and 4/21 (19%) vasculitis confined to glomerulus. In every patient the infection was pulmonary or pleurical. Mycobacterial infection preceded the vasculitis event by almost 10 years with two exceptions:

- a female patient who had a TBC relapse 2 years before developing the micropoliangioitis; and

- a male patient in which the renal bioptical diagnosis of vasculitis and Koch identification on sputum failed at the same time and later the microbiological culture resulted positive too.

One patients was also affected by silicosis.

3.1.3. Study III

The ANCA research gave a negative result in all but two of the considered patients (Table 4). In both cases, the positive results disclosed the presence of P-ANCA at a moderate degree (37 and 29 U ml^{-1}, respectively).

3.2. Experimental study

3.2.1. Study IV

3.2.1.1. OFR production. Human PMN incubated with crystalline Silica (Min-U-sil) produced a dose-dependent burst of OFR, which is maximum at 10 min of incubation (Fig. 2). Both at 10 and 15 min of incubation OFR production was higher than that induced by yeast. However, diatomaceous earth produced a small increase of OFR production only at high doses (100 μg ml^{-1}) at 15

Table 4
ANCA positive results in cases and controls: distribution

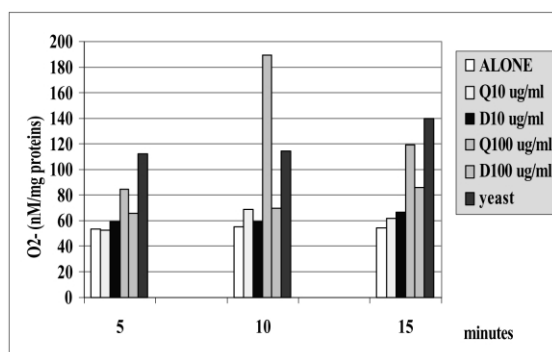| Subjects	P-ANCA (anti-MPO)		C-ANCA (anti-Pr3)	
	Positive (> 20 U ml^{-1})	Negative (< 20 U ml^{-1})	Positive (> 20 U ml^{-1})	Negative (< 20 U ml^{-1})
Cases				
Professional exposure				
Silica ($n = 44$)	1	43	0	44
Asbestos ($n = 20$)	1	19	0	20
Total ($n = 64$)	2	62	0	64
TBC infection ($n = 16$)	0	16	0	16
Controls				
Geriatric patients ($n = 65$)	0	65	0	65

Fig. 2. Oxygen free radicals production by polymorphonuclear (PMN) exposed to different types of silica. O_2^- (superoxide anion) production is expressed as nanomoles of cytochrome C reduced per milligram of protein. Protein content of PMN is measured according to the Lowry technique. The effect of quartz (Q) 10 and 100 μg ml^{-1} and diatomaceous earth (D) 10 and 100 μg ml^{-1} was recorded at 5, 10 and 15 min of incubation and compared with cellular culture incubated alone (zero stimulus) or with yeast (maximum stimulus).

min of incubation and always less than that observed for crystalline silica and yeast (Fig. 2).

3.2.1.2. Cytokine detection. There was a dose-dependent production of IL-12 by PMN stimulated with crystalline Silica (Min-U-sil). Doses as low as 10 μg ml^{-1} induced an IL-12 production more than 10-fold higher in comparison to basal value (IL-12 < 5 pg ml^{-1} in supernatant of unstimulated PMN). Doses of 100 μg ml^{-1} induced an IL-12 production as high as (50 pg/ml) that observed when yeast (55 pg/ml) and LPS (45 pg ml^{-1}) were used as positive controls. Diatomaceous earth produced a smaller increase of IL-12 production (10 pg ml^{-1}) only at high doses (100 μg ml^{-1}). Preliminary results with monocyte showed that both crystalline and amorphous silica induced an increased production of IL-12, which seems higher when aerosil (synthesized amorphous silica) is employed.

4. Discussion and conclusions

The results we obtained seems to confirm a significant correlation between silica exposure and renal vasculitis. These are consistent with those

reported in literature, having the advantage of deriving from a wider case record in comparison with the only two case-control studies published up to now (Gregorini et al., 1993; Nuyts et al., 1995a,b) and strongly suggest the opportunity of confirming this evidence in a larger samples of patients. One limit of our study was the type of design (retrospective case/controls analysis) The choice of a case-control retrospective study was required by the low incidence of the pathology examined (Nuyts et al., 1995a,b). As one of the main 'confusing factors' is the possibility that cases and controls do not belong to the same population, special attention was, therefore, drawn to the fact that cases and controls must be recruited in the same region of residence, in order to have analogous environmental and working conditions. However, to avoid bias due to still relatively low number of cases and retrospective design of study, we planned to extend this research on a national basis with recruitments of cases and controls from other nephrologic units.

Another project coming from the results of this study will be that of extending the use of a structured questionnaire, analogous to the one used for the occupational exposure, also to the daily clinical habits. In fact, as far as women are concerned, most patients do not show any sign of exposure to pollutants, with regards to their job typology. However, they could be subject to insidious silica exposure, seeing that this pollutant may be found in some types of detergents, medicaments and cosmetics (e.g. salve and aerosol) (Koeger, 1994). For this reason, the gathering not only of a detailed job history but also of habits and life-style may be very important.

A further hint for research is given by the confronting of silica and asbestos. In fact unlike silica, the exposure to asbestos in controls resulted unexpectedly higher than in cases. As pulmonary cellular basement membranes share some constituents with renal glomerular basal membranes, it could be suspected that the relationship between silica and renal vasculitis might be mediated by specific damage to pulmonary cells due to inhaled silica. In this case, similar relationships would also be observed after damage to pulmonary cells produced by another inhaled toxicant

such as asbestos. As this is not the case, it seems justified to speculate on chemical/physical or other effects which are specific for silica (Wagner, 1997).

Using only epidemiological data, it is difficult to trace back the type of silica to which the patient was exposed. It is possible that its crystalline form is present in all job typologies, but in varying quantities. It is certainly higher for miners, sandblasters and sandpit workers, while farmers (13% of patients affected by vasculitis and 28% of exposed) are not only exposed to the quartz present in the ground, but also to silicon-containing compounds including grain dust. Even if exposure is for this working typology of minimal frequency and intensity, its duration is higher. It is, therefore, possible to hypothesize that the silica form, the duration and entity of exposure are responsible for the triggering of a silica-induced autoimmune mechanism.

Recently an aberrant Th1 response was supposed to be associated with the granulomatous inflammatory lesion in WG (Lùdvìksson et al., 1998). Th1-type immune responses are characteristic of cell-mediated immunity, while Th2-type responses are associated with humoral immunity and allergic phenomena. Preferential induction of the Th1-type or Th2-type response has profound immunologic consequences, either pathogenic or protective according to the circumstances (Biancone et al., 1996; Del Prete, 1998). IL-12, a heterodimeric cytokine mainly produced by monocytes and macrophages, promotes Th1-type cytokine production, such as IFN-γ, and inhibits Th2-type cytokine production. Monocytes isolated from patients either with active WG disease or with inactive WG disease produce greatly increased levels of IL-12 when stimulated. This finding implies that the increase in IL-12 production is not a secondary effect to the inflammatory process itself, but rather a primary feature of WG. The cytokine studies help to postulate a series of pathologic events responsible for vasculitis: the initial event is an exposure to an environmental Ag(s) that induces an excessive macrophage IL-12 response and leads to unbalanced T cell response characterized by overproduction of IFN-γ and TNF-α. This is followed by the establishment of a granulomatous inflammation via changes in cellular adhesion and activation of monocytes and T cells. The final event is inflammation-induced tissue breakdown with the release of intracellular materials from infiltrating cells, such as proteinase-3, which causes further autoimmune responses that amplify the primary lesion. The environmental Ag(s) capable of initiating this cascade is presently unknown.

In relation to TBC analysis we conclude that the case-control study results (although the statistical sample was small) seem to confirm the possibility of a correlation between TBC and ANCA associated vasculitis. It is intriguing the fact that they may have common immunological mechanism characterized by the activation of cell-mediated immunity and by granulomatosis reaction. For this reason, even if we had not found a stronger correlation between TBC and WG, it could be interesting to try to evaluate this relationship on a wider sample. However, it is important to underline that the only patient who had both contemporary TBC and vasculitis was affected by WG. Trying to hypothesize eventual ethiopatogenetic mechanism, we suppose that mycobacterial is a chronic stimulus for an immunogenic reaction able to cause a vasculitis process. On the other hand, it could exist a common predisposing factor to both diseases (TBC and vasculitis) such as a macrophagical characteristic or a linfochinical situation, Th1 mediated (Rook and Stanford, 1996).

Lastly, as to the analysis concerning the frequency of positive ANCA test among subjects with occupational exposure or previous/present TBC infection, we planned to collect a wider sample population and to follow the subjects with positive results.

Generally speaking, our study provided some new insights to lightening the puzzling network linking silica, TBC, vasculitis and ANCA, allowing to focus on topics which merit additional studies to further speculate on these associations in terms of epidemiology and pathogenesis.

We conclude that silica exposure may be related to the occurrence of vasculitis with renal involvement, also in the absence of overt silicosis,

but we want to stress that another unexpected conclusion of our work is the high frequency of occupational exposure in patients we assumed as controls, who are suffering from other types of renal diseases. This observation merits further study in larger samples.

What has to be underscored is that a thorough occupational history is critical in the evaluation not only of patients with otherwise unexplained renal insufficiency, but also of patients with recognizable cause of renal diseases. In fact, nephrotoxic substances not only causes renal diseases directly, but they can also destroy renal reserve capacity, potentially placing those people with additional risk factors, such as glomerulonephritis, diabetes, hypertension, cardiovascular disease and genetic predisposition, at greater risk.

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