

Inflammation Markers in the Evolution of Asbestosis

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Asbestos structure and composition contribute to the lung injury and to the inflammation induced by this natural fiber. The result of this study is that neutrophil to lymphocyte ratio correlates significantly to timing of progress of the radiological lesions in the evolution of patients with asbestosis, followed for 5 years. If confirmed in larger studies, this could become a cost-effective biomarker for the asbestosis evolution.

Key words: Inflammation markers, asbestosis, ENT (ear, nose and throat) examination

Despite asbestos banning from industrial settings in many countries, the asbestos epidemic is far from finished. The current estimation is for almost 107.000 death/year from former occupational exposure to this mineral. [1] All types of asbestos fibers are now considered fibrogenic and carcinogenic, but the latency is variable, reaching sometimes a period of more than 50 years of lung retention. [2] The type of fiber, the intensity and length of the exposure, the presence of other particles, fumes or vapours in the workplace and several genetic factors explain most of these differences. Asbestos fibers of medium and high length resist to macrophage digestion and persist for a long time in the lung. The pathogenic mechanisms initiated by the retention of the asbestos fibers inside the lung and pleura involve the inflammasome, neutrophils recruitment and activation, cytokines and reactive oxygen species generation and fibrosis. [3]

Experimental part

A retrospective analysis of the asbestosis cases evaluated yearly in the Clinic of Occupational Diseases of the Colentina Clinical Hospital, during 2014-2019, was performed. Cases with an acute infection were excluded. Data extracted from the medical files included: age, gender, smoking habit, co-morbidities, blood count, erythrocyte sedimentation rate (ESR), spirometry values and radiological findings. The radiographies were classified as recommended by the International Labour Organization. [4] The occupational exposure data referred to the job title, type of activity and occupational sector; the exposure time was defined as number of years of occupational contact with asbestos and the retention time as duration (in years) from the first documented exposure until the time when the examination took place. The smoking habit was expressed in number of pack-years. According to the number of pack-years, smokers and ex-smokers were classified as: light (<10 pack-years), moderate (11-20 pack-years) and heavy (>21 pack-years).

Because asbestos exposure was associated with increased risks of laryngeal cancer/sinonasal tumors, we decided to perform a full ENT examination (with head computed tomography scan) to all subjects.

Spirometry at admission was performed with a Jager/Viasys Pneumotachograph (CareFusion, Germany). The

forced vital capacity (VC) and of the forced expiratory flow in the first second (FEF₁) data were expressed as percentage from the reference. The neutrophil to lymphocyte ratio (NLR) was calculated from the blood count at admission in 2014 and after the 5 years of follow up. The evolution of the lung disease was assessed by comparing the X-ray interpretation from 2014 to the one in 2019. Based on this, the patients were divided in 2 groups: patients with signs of radiological progression (group 1) and patients with no radiological changes during this time. Comparison of demographic, occupational characteristics, lung function and of the inflammatory status assessed by the NLR and ESR were performed.

Data were processed with SPSS using the Statplus for Mac software, 2016, v6 (Walnut, US). The variables are presented as mean and standard deviation (StDev) or medians and range, according to the normality of the distribution. Comparison between groups was done using Anova for the normal distributed variables and with Mann-Whitney test U test for the others. The χ^2 test was used to compare the distribution of the qualitative variables. A threshold of 95% was selected for the statistical significance.

Results and discussions

Of the total of 44 patients that were followed in the clinic during the last 5 years, 2 were finally excluded due to the presence of an acute infection. The average age was 52.74 years. The gender distribution showed a predominance of men (27 versus 15 women). This was an expected finding, as most of the jobs were manual industrial activities. However, it has to be underlined that in comparison to western countries, the proportion of women is very high [5]. In the past, more women were occupationally exposed to asbestos in Romania, working in jobs traditionally done by men. Half of the patients were locksmiths (n=11) and electricians (n=10) in industries with asbestos exposure or in construction sites. The others were joiners (n=3), technicians (n=3), chemist operators in asbestos factory (n=2), ferodo workers (n=2), moulders (n=2), plumbers (n=2), turners (n=2); there was one craneer, one forger, one steel worker, one storage worker and an asbestos weaver.

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Table 1
MAIN CHARACTERISTICS OF THE STUDY GROUPS

Characteristic	Group 1	Group 2	Total	P
Age (average \pm standard deviation)	53.95 \pm 7.76	51.52 \pm 6.14	52.74 \pm 7.02	0.27
Exposure time* (average \pm standard deviation)	22.48 \pm 6.15	21.52 \pm 7.58	22 \pm 6.84	0.66
Retention time* (average \pm standard deviation)	33.81 \pm 8.31	33.62 \pm 6	33.71 \pm 7.16	0.93
NLR 1* (median and percentile 25%-percentile 75%)	2 (1.57-2.65)	2.03 (1.84-2.19)	2.02 (1.76-2.63)	0.73
NLR 2** (median and percentile 25%-percentile 75%)	2.41 (2.06-3.14)	1.81 (1.52-2.3)	2.12 (1.65-2.74)	0.04
ESR 1* (average \pm standard deviation)	15.84 \pm 10.66	23.28 \pm 16.67	15.93 \pm 9.44	0.96
ESR 2** (average \pm standard deviation)	23.29 \pm 16.67	19.45 \pm 13.95	21.41 \pm 15.37	0.43

* = initial, at the first evaluation; ** second evaluation (after 5 years of follow up).

Numerous ways were developed to estimate the occupational exposure (self assessment, job titles, lengths of exposure, specific occupational task, job-exposure matrix) [5,6]. In our cases, exposure assessment was based on the documentation provided by the past employer and on expert evaluation of the job risk. The average exposure time and the average retention time were more than 20, and 30 years, respectively, in line with the slow progression of the lung fibrosis after asbestos exposure (table 1).

There were 21 cases assigned to the group 1, showing radiological progression, and 21 cases to the group 2, with no differences in age, exposure time and retention time between the 2 groups (table 1). Even if there was a slightly higher percentage of men in group 1, the gender distribution among the 2 groups was not statistically significant ($\chi^2=2.8$, $p=0.094$). There was also no difference in jobs distribution between the 2 groups ($\chi^2=98.76$, $p=0.088$).

Few current smokers were found in this sample of patients ($n=5$), 4 in the progressive disease group and 1 in the non progressive disease group. The number of nonsmokers was equal in both groups (10 in each group). If there is a multiplicative or an additive effect between asbestos and smoking in increasing lung cancer risk is still debated in the literature; the risk is also high for the nonsmokers [7].

To the best of our knowledge, there are no published data regarding an additive effect on smoking on the asbestosis incidence, but a certain skepticism to attribute the fibrosis to asbestos exposure in heavy smokers has been raised from confronting radiological interpretation with pathological findings [8]. This is also the case for other asbestos-related diseases, such as mesothelioma, when not all elements for the recognition of the occupational exposure are present.[9] Both asbestos related diseases and smoking addition show genetic influence.[10,11] In what concerns asbestosis, the development of lung fibrosis is associated with the nodd like receptor 3 (NLRP3) rs35829419 variant allele, while the transforming growth factor (TGFB1) rs2241718 variant allele with decreased risk [12]. Smoking induced lung fibrosis has similar radiological findings with asbestosis, although the histology is distinguished: the characteristic pattern of asbestosis is the initial localization of fibrosis in the bronchiolar wall and peribronchiolar area of the subpleural region of the lung and the presence of the asbestos bodies; fibrosis progressively extends into alveoli, further from the bronchiole. In the more advanced stages, fibrosis bridges between adjacent respiratory bronchioles and creates the aspect of the honeycomb fibrosis. Fibroblast foci are rare, but at least mild fibrosis of the visceral pleura is present.[13]

The smoking induced fibrosis has also a centriacinar development, but lacks the honeycomb aspect, the pleural involvement and the fibroblastic foci; in contrast, it associates the other pathological consequences of smoking exposure: bronchiolitis and emphysema.[14] In our study, we could not attribute progression to differences in smoking habits, as the distribution between ever-smokers (current and past) and non smokers was not statistically significant ($\chi^2=0.810$, $p=0.368$). There were also no difference in the distribution between light/moderate/heavy smokers among the two groups ($\chi^2=2.436$, $p=0.487$). No sign of ENT malignancy was found.

Due to the large number of missing data, we were not able to calculate differences in lung function parameters at the initial time considered in this analysis (in 2014). For 2019 we have a better picture on the lung function of these patients: except for one patient in group 1, a heavy smoker with obstructive lung disease and a FEF₁ of 41.5% from the predicted value, 8 patients had a mild reduction of the values of the CV% (less than 80% of predicted, but higher than 70% of the predicted one), 3 in group 1 and 4 in group 2. The rest of them ($n=34$) had normal spirometric values. These findings are consistent with the radiological findings that did not show in any of these cases an extensive, massive fibrosis. The average value of VC (average = 89.80%, StDev = 13.96) was similar but the FEF₁ (average = 93.01%, StDev = 15.57) was slightly higher than the average reported in a meta-analysis based on data from 9921 workers exposed to asbestos.[15] We have previously shown that spirometry values are not an early change in pneumoconiosis, even when symptoms and quality of life is impaired [16]. In fact, reduction in lung function values (VC, FEF₁) persists after the exposure to asbestos is stopped [17] and we reasonably expect the lung function impairment of these patients to further aggravate. Diffusing capacity for carbon monoxide, a more sensitive marker of interstitial disease, is declining in asbestosis more rapidly than in the normal population, independent of the smoking status [18].

Concerning the inflammatory markers, no difference was noticed between the NLR and ESR values in the 2 groups ($p=0.734$ and $p=0.958$, respectively), when recorded at baseline (in 2014) (fig 1). We interpret this in the context of the low level of inflammation and the slow progression that are the main distinctive histological features of this particular lung fibrosis [13]. There is evidence that asbestos has a direct mechanical injury, generates reactive oxygen species on its surface, alters the cellular signal transduction pathways and silences the

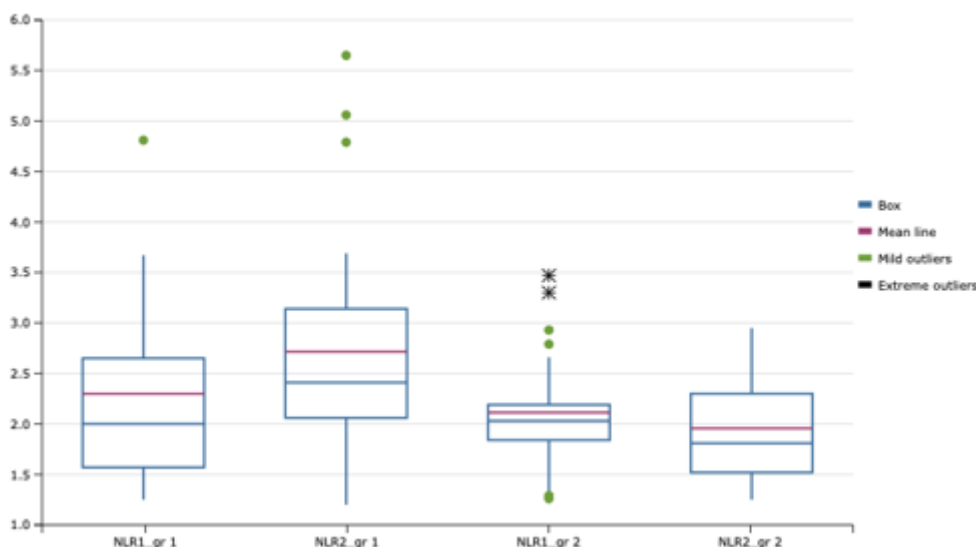


Fig 1. Distribution of the NLR values

Legend: NLR1_gr1= initial value of neutrophil to lymphocyte ratio in group 1; NLR2_gr1 = value of neutrophil to lymphocyte ratio in group 1 after 5 years from the initial evaluation; NLR1_gr2= initial value of neutrophil to lymphocyte ratio in group 2; NLR2_gr2 = value of neutrophil to lymphocyte ratio in group 2 after 5 years from the initial evaluation

tumours suppressor genes, while activating the pro-oncogens. [19]. Alveolar macrophages are the first cellular line of the defense mechanism after asbestos exposure; for fibers with very short lengths, this mechanism is rather efficient, but for longer fibers (particularly if longer than 20 mm), the macrophages clearance is limited and persistence in the lung is longer. The capacity of asbestos to generate ROS directly from the surface of the fiber or after being ingested by neutrophils or alveolar epithelial cells is the signal to activate the innate response immune system and the fibrogenic process [20] and maintains the chronic inflammatory state. The activation mechanism initiated by asbestos is supposed to require actin-mediated cellular uptake and lysosomal disruption; the cathepsin B released from lysosomes triggers the NLRP3 [21]. Others have demonstrated that fibers remaining on the surface generate ROS, a danger signal detected by the NLRP3 [22]. Through activation of caspase 1, IL-1 β and Tumor Growth Factor- β production is enhanced, neutrophil attraction increases in sterile lung inflammation [23]. What makes the asbestosis retention in the lung to evolve after a such a long latency to the radiological characteristic opacities is not known, but it probably the result of the persistent lung injury. In this study we searched for a role of the neutrophils in this evolution (fig 1).

We could not find the initial NLR to be correlated with the radiological progress ($p=0.73$), but this pattern changed after 5 years: in 2019, the NLR values were significantly associated with progression of the radiological signs, as following: the average NLR was 2.71 and the median equaled 2.41 in group 1, while the average NLR was 1.96, with a median of 1.81 in group 2. This difference was statistically significant (Mann-Whitney, $U=303.5$, $p=0.04$). Despite the difference between the NLR, the ESR did not differ between the two groups (average ESR in group 1=23.29, ESR in group 2 = 19.45, $p=0.43$).

The neutrophil role in the asbestos lung has been suggested by the cellularity of the bronchoalveolar lavage, in which macrophages are prevalent, but there is always a moderate number of neutrophils [24]. Neutrophils are primed *in vitro* by exposure to asbestos, releasing myeloperoxidase, that is captured by epithelial and bronchial cells cultured from human lung tissues of patients with asbestosis [25]. Inside these cells, myeloperoxidase

promotes hemoxygenase 1 and the DNA breakage, eventually leading to a significant cell damage [26]. Asbestos is also able to initiate the formation of neutrophil extracellular traps with extracellular DNA and pro-inflammatory proteases release damaging surrounding tissue [27]. In view of these mechanisms of lung disease, investigating neutrophils in asbestosis is necessary.

In silicosis, we have reported that NLR is [28] a predictor for silicosis evolution, when the major possible confounders were excluded (smoking and any chronic or acute lung disease). In this study, we had excluded only the acute lung disease, due to the limited number of patients. This does not allow us to suggest the same conclusion for asbestosis; however, the simultaneity of the NLR increase noticed when additional lung and/or pleural opacities were observed is important to report. As NLR may reflect the active inflammation in asbestos patients, it is worth to be further investigated.

Conclusions

The results suggest that NLR is not a prognostic marker for the asbestosis evolution, but could be an inflammatory sign associated with the radiological progression. At the same time, we must mention that the information provided by the ENT examination has not shown a causal association between asbestos exposure and laryngeal cancer or sinonasal tumors, in our study.

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Manuscript received: 14.12.2018