In situ analysis by SEM-EDX spectroscopy of 10 sarcoidosis cases from MINASARC study

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I. Introduction

MINASARC is a case-control study (20 cases and 20 healthy volunteers) designed to assess the inorganic 'exposome' by comparing the mineralogical analysis (MA) of Broncho-Airvelar Lavage (BAL) fluid by transmission electron microscopy (TEM). One of the secondary objectives of this study is to compare those results with an in situ analysis of some paraffin-embedded biopsies by scanning electron microscopy (SEM). We report data from 10 sarcoidosis patients corresponding to the 10 biopsies available for analysis.

II. Material and methods

A. Patients (Table 1)

Criteria for inclusion and exclusion of Sarcoidosis patients

Inclusion: suspected sarcoidosis stages 1-4; age 20-50; having signed an informed consent; being admitted to an endoscopic examination with BAL; without probable causal factor already identified during the usual patient interview; accepting to complete a professional and environmental questionnaire once sarcoidosis is confirmed.

Exclusion: refusing the terms of the informed consent; refusing to complete a professional and environmental questionnaire; non-completion or failure of BAL.

Table 1: Demographic and clinical characteristics of the sarcoidosis patients. PN: patient number; Smo Sta: smoking status (0: < 5 PY; 1: 5 to 10 PY; 2: > 10 PY); Stage: radiologic stage of the disease; EBUS: endobronchial ultrasound; TBNA: transbronchial needle aspiration; BB: bronchial biopsy; SAGB: salivary gland biopsy; LB: liver biopsy; SB: skin biopsy.

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B. MA by Electron Microscopy

1. Analysis of BAL fluid in TEM

The BAL’s MA was performed by transmission electron microscopy (TEM, Jeol 4000 EX, operated at 120 kV) on a device equipped with a CCD camera (Gatan Orius 690) and an X-ray emission detector (Jeol JED-2300). EDX spectra were acquired of a 5 μm thick unstained section deposited on a silicon substrate. The particles were identified and classified using the Jeol JED-2300. EDX spectra were acquired of the particles identified by TEM and classified according to their chemical composition.

2. Analysis of histological section in SEM (Fig.1)

A first HES stained histological section was observed under an optical microscope (ZEISS Axioscope A1). A second 5 μm thick unstained section was deposited on a carbon disc with a diameter of 25 mm. This section was intended for observation by scanning electron microscopy (JEOL JSM-6010PLUS/LV) equipped with an Oxford X-Max micro-analyzer 50 μm². The analysis of the particles was carried out under the following conditions: backscattered electrons; 20KV intensity; 12mm working distance. Spot size 42; Pressure 20 Pa; Magnification x5000. At this magnification one field corresponds to 0.05 mm². For each sample, 5 fields were analyzed.

III. Results

A. Results of the MA (Table 2)

Table 2 shows the result of the MA on the BAL fluids and on the paraffin-embedded biopsies from the 10 sarcoidosis patients. For the BAL fluid, the ranking was based on the 39 individuals included in the study (one healthy subject was excluded for MA). For the biopsy, the dust level was calculated according to the average number of particles per field. The particles analyzed by EDX were classified according to their chemical composition.

Table 2: Results of the mineralogical analysis in electron microscopy. Level of particles: 1: < 10 particles per field; 2: 10 to 50 particles per field; 3: > 50 to 100 particles per field; 4: > 100 particles per field. P: phosphor; Ca: calcium; Si: silicon; O: oxygen; Al: aluminum; Na: sodium; Fe: iron; Mg: magnesium; Ti: titanium; Ni: nickel; Cr: chromium; Cl: chlorine. The chemical composition of the analyzed particles is reported in the elements detected on the EDX spectrum. Considering the exposed particles, “SO” is interpreted as being silica, “Pca” as calcium phosphate probably of endogenous origin, “BAA” as aluminium oxide, “Pac” as sodium phosphate, “TiO” as titanium oxide, “CompT” as particles composed of titanium, “FeCrNi” and “FeCr” as steel, “TiNi” as titanium and nickel alloy. “CaO” as calcium oxide, “Cl” as chlorine particles.

B. Illustration of patients n°1 and 4 (Fig. 2 and 3)

Patient N°1 was a 41-year-old man of French origin, public relations worker, and suffering from stage 2-sarcoidosis. The mineralogical analysis of the BAL fluid revealed a high rate of chromium particles compared with the 19 healthy volunteers included in the MINASARC study. Fig. 2 shows the mineralogical analysis of the lymph node biopsy performed by endobronchial ultrasound trans-bronchial needle aspiration (EBUS/TBNA). Optical microscopy revealed the presence of opaque and birefringent particles. Electron microscopy showed the presence of numerous inorganic particles (TiNi and FeCrNi) gathered into a cluster.

Patient N°4 was a 28-year-old woman of French origin, non-smoker, esthetician manicurist and affected with stage 1-sarcoidosis. The mineralogical analysis of the BAL fluid revealed a high level of sulfur compounds and silica particles compared with the 19 healthy volunteers included in the MINASARC study. Fig. 3 shows the mineralogical analysis of the lymph node biopsy performed by EBUS/TBNA. Optical microscopy revealed the presence of opaque and birefringent particles. Electron microscopy showed the presence of numerous inorganic particles (SiO and FeCrNi).

IV. Discussion and conclusion

MA-SEM on tissue sections is feasible and can give interesting results when compared with the MA of the BAL fluid and the patient’s occupational and environmental exposure. In patient N°4, a manicurist, it identified silica particles in the lymph node and a high level of calcium oxides in the BAL fluid. As mentioned [1], these silica particles may arise from the powder used to polishing the nails before the methacrylate polymer application. Such an exposure may be considered to be involved in the granulomatosis affecting the patient. In patient N°10, a safety worker, steel particles were identified in the biopsy associated with a steel overload in the BAL fluid. This patient has worked as a plumber (less than one year). During this period, he carried out welding and drilling on metal products and paints. In this patient, exposure to metallic particles may be suspected of being a cause of granulomatosis in reaction to steel.

For EBUS/TBNA cases, we identified FeCrNi and TiNi particles compatible with metal particles released from the needles used for TBNA, as already reported [2].

The size of the biopsy is an important factor for the in situ MA. In order to have an adequate sensitivity of the analysis, it is necessary that the surface of the histological section be greater than 0.5 cm².

The in situ MA with SEM that we performed neither allows us to analyze all the particles present on the histological section nor to detect nanoparticles. This is feasible with the development of an in situ analysis using the Laser Induce Breakdown Spectrometer (LIBS) [3].

The in situ MA with SEM on paraffin embedded biopsies allows to determine the chemical distribution of particles observed in the preserved structures of tissues. It offers additional information on the patient’s exposure to inorganic dusts and, for some patients, it helps to shift the diagnosis toward a mineral dust-induced granulomatosis.

Calcium phosphate particles were observed in 7 out of 10 patients. This compound has a probable endogenous origin and could be related to the onset of the pathology as described for sarcoidosis [4,5].