Identification of metal sensitization in sarcoid like metal exposed patients by Melisa® lymphocyte proliferation test

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ABSTRACT

Background: Metal dusts and fumes exposure occurs in many occupational settings. The lung can respond to the inhalation of these metals in a variety of lung diseases such as parenchymal lung fibrosis and granulomatous lung disorders.
Aim: to identify sensitization to metals that cause granulomatous lung disease other than Beryllium (Be).
Methods: 8 sarcoid like metal exposed patients (SA-ME pts) and 2 CBD (Chronic Beryllium Disease) pts underwent Beryllium Lymphocyte Proliferation Test (BeLPT) and Melisa Lymphocyte Proliferation Test (MeLPT). 9/10 biopsies were tested for metal content by Scanning Electron Microscope (SEM).
Results: Metal selection for MeLPT was done according to SEM results and exposure history. In 7/10 pts metal sensitization was identified by MeLPT. Concordance for BeLPT results was confirmed in 1/2 CBD pts but Be new sensitization was identify in one SA-ME pt.
Conclusion: MeLPT is an effective additional tool to identify sensitization to metal in Sa-ME pts to identify metal sensitivity to metals other than Be.

	Diagnosis	Metals tested by SEM	Metals tested by Melisa	MeLPT	BeLPT
1	SA; NCG(-)	Al, Cu, Si	Al, Cr, Si, Ti, Hg	Ti(+)	Be(-)
2	SA; NCG (+)	Fe, Ti, Al, Si	Al, Si, Ti	(-)	Be(-)
3	SA; NCG (+)	Si, Cr, Ni, Fe	Cr, Fe, Ni, Si, Ti, Be	Ti(+)	Be(-)
4	SA; NCG (+)	Si, Cr, Ni, Fe, Mo	Cr, Au, Hg, Pa, Ti, Ni, Mo	Ti(+) Pa(+)	Be(-)
5	SA; NCG (+)	Au, Zn, Si, Al,	Al, Au, Hg, Pa, Si, Ti, Zn, Be	Hg(+)	Be(-)
6	SA; NCG (-)	Al, Ag, Cu	Ag, Cu, Al	(-)	Be(-)
7	SA; NCG (+)	Al, Si F Zn	Hg, Ni, Si, Ti, Zn, Pb, Ti	Ti(+) Pb(+)	Be(-)
8	SA; NCG (+)	Al, Si Fe	Al, Au, Fe, Si, Ti, Be	Ti(+) Be(+)	Be(-)
9	CBD NCG (+)	(-)	Fe, Ni, Ti, Be	(-)	Be(+)
10	CBD NCG (+)	Al, Si Cr, Fe, Ni	Al, Cr, Fe, Ni, Si, Be	Si(+)Fe (+)Cr(+) Ni(+) Be(+)	Be(+)

BACKGROUND

Sarcoidosis (SA) is a systemic disorder of undetermined etiology, defined histopathologically by non-caseating granulomas but remains an enigma for several reasons. Presence of granulomatous inflammation is a nonspecific finding, being also present in mycobacterial or fungal infections, hypersensitivity pneumonitis and associated with inhalation of metal dust or fumes. Metal dusts and fumes exposure occurs in many occupational settings [1]. The lung can respond to the inhalation of these metals in a variety of lung diseases such as parenchymal lung fibrosis and granulomatous lung disorders. In recent years the greatest progress in our understanding of pneumoconiosis, other than those produced by asbestos, silica and coal has been in the arena of metal induced parenchymal lung disorders.

HYPOTHESIS

Our hypothesis is that metal exposed patients with sarcoid like reactions are misdiagnosed as SA .

AIM

To identify sensitization to metals that cause granulomatous lung disease other than Beryllium (Be).

STUDY POPULATION

Table 1: *List of Patients included in the study*

Number	Gender	Age (yrs)	Occupational exposure
1	Male	68	Copper industry
2	Male	70	Military Industry
3	Male	61	Metal Worker
4	Female	63	Jewelry industry
5	Male	58	Aircraft Industry
6	Male	70	Welder
7	Male	63	Welder
8	Male	63	Military Industry
9	Male	45	Dental technician
10	Male	50	Dental technician
11	Female	40	Dental technician
12	Female	45	Teacher
13	Female	35	Silicone Implants
14	Female	40	Silicone Implants

METHODS

Clinical Assessment

All subjects gave written informed consent to be enrolled and the studies were ethically approved by the Tel-Aviv Sourasky Medical Center Institutional Ethics Committee. Subjects completed demographic, clinical and occupational questionnaires.

Beryllium Lymphocyte Proliferation Test (Belpt)

BeLPT was performed within 24 hours of venipuncture on all subjects according the method of Mroz et al [2]. Results were expressed as a stimulation index (SI), which is the ratio of the counts per minute of radioactivity in cells stimulated with beryllium salts divided by the counts per minute for un-stimulated cells. The SI normal ranges are laboratory-dependent and based on the mean peak SI plus 3 standard deviations (SDs) for unexposed subjects. SIs higher than these values were considered to be elevated. An abnormal BeLPT was required to have two or more elevated SIs occurring at any of the BeSO4 concentrations tested. An SI >2.5 was considered abnormal.

Melisa Lymphocyte Proliferation Test (Melpt)

Blood samples were sent to Laboratoire MGDSA (Geneva-Switzerland MeLPT LTT-MELISA®) and collected into vacutainer tubes with polystyrene beads (Becton Dickinson, UK), defibrinated by shaking and diluted 1:1 in a modified culture medium RPMI 1640 with 10 mM Hepes (Gibco BRL) and gentamycin (Krka, Yugoslavia). Mononuclear cells were separated by centrifugation for 30 minutes at 600 g on a Ficoll-Paque gradient (Pharmacia, Uppsala, Sweden), followed by washing.

Cells were resuspended in 20% autologous inactivated serum and incubated for 30 minutes at 37oC and in 5% CO2 atmosphere in culture flasks (Costar, USA). This procedure results in partial depletion of monocytes from lymphocyte suspension. After incubation, cells were resuspended in complete medium containing glutamine and 10% inactivated autologous serum in concentration 1x 106 cells per ml. One ml of cell suspension was added to each well in 48-well tissue plates (Costar, USA) pre-coated with metal salts. Stock metal solutions were prepared by dilution of metal salts (Table 2) with sterile filtered tissue water (TW) and further diluted to working solutions at the time of plate preparation [3]. Three wells with 100 µl TW without metals were used as negative controls. Pokeweed mitogen (PWM, Sigma; in concentration 10 µg/ml) was used as positive control. After 5 days incubation at 37oC and 100% humidity, 600 µl of cell suspension from each well was transferred to a new plate and 111 kBq of methyl3H thymidine (UVVVR, Prague) was added. After 4-hour incubation, samples were harvested using an automatic cell harvester (Inotech, Switzerland) and the radioactivity incorporated into DNA of cells was counted on a Microbeta counter (LKB/Wallac, Finland). Lymphocyte stimulation in metal-containing cultures was expressed as stimulation index, SI:
SI = cpm (counts per minute) in experimental cultures divided by mean cpm in control cultures. SI equal or more than 2 indicates positive response (Table 3).

Table 2 : *chemical compounds used for MeLPT*

Number	Compound Name	Formula
1	Aluminium chloride.	$AlCl_3$
2	Titanium dioxide	TiO_2
3	Titanium sulphate	$Ti(SO_4)_2$
4	Chromium chloride	$CrCl_3$
5	Sodium silicate solution	Na_2SiO_3
6	Gold sodium thiosulfate	$AuNa_3O_6S_4$
7	Mercury (II) chloride	$HgCl_2$
8	Iron oxide	Fe_2O_3
9	Molybdenum chloride	$(MoCl_3)_2$
10	Nickel chloride	$NiCl_2$
11	Palladium chloride	$PdCl_2$
12	Copper sulphate	$CuSO_4$
13	Silver acetate	$AgCOOCH_3$
14	Beryllium sulfate	$BeSO_4$
15	Tin chloride	$SnCl_2$
16	Platinum sulfate	$PtSO_4$
17	Cobalt chloride	$COCl_2$
18	Mangan chloride	$MnCl_2$
19	Tungsten chloride	WCl_6

Mineralogical Studies

Mineralogical studies were performed on the paraffin block of the lung biopsy. A sample from a paraffin block was processed for electron microscope studies by embedding it in Epon. Thin sections were cut and slightly contrasted by uranyl acetate The chemical composition of selected specimens was investigated by counting 500 particles (>0.8 mm in diameter) by X-ray analysis using a JEOL 840 SEM equipped with a Link 10,000 energy-dispersive system (EDS). The spectrometer of the EDS system separates the elements according to energy rather than wavelength. In addition, a Petrographic microscope was used to identify the minerals (Fig 1).

Fig. 1a,b,c: *Representative sem analysis in patient*

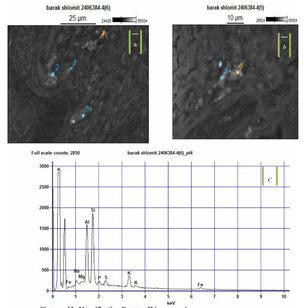


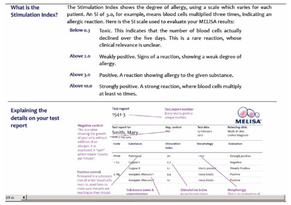
Fig 1a and b: Magnification X 4500 of biopsy specimen.
Fig c: X-ray spectra of representative particles identified

Fig 1a and b: Magnification X 1500 of biopsy specimen.
Fig c: X-ray spectra of representative particles identified

Table 3: *Representative MeLPT testing*

2. Cr	Chromium I	1.8	-	Negative
	Chromium II	2	-	Weakly Positive
	Chromium III	2.6	-	Weakly Positive
3. Hg	Inorganic Mercury I	1.9	-	Negative
	Inorganic Mercury II	1.1	-	Negative
4. Fe	Iron I	1.7	-	Negative
	Iron II	1	-	Negative
	Iron III	1	-	Negative
5. Ni	Nickel I	8.6	-	Positive
	Nickel II	2.2	-	Weakly Positive
6. Si	Silica I	35.9	-	Strongly positive
	Silica II	1.6	-	Negative
	Silica III	1.4	-	Negative
7. Ag	Silver I	0.3	-	Negative
	Silver II	0.3	-	Negative
8. Sn	Tin I	1.7	-	Negative
	Tin II	1.3	-	Negative

Lymphocyte stimulation in metal-containing cultures was expressed as stimulation index, SI: cpm (counts per minute) in experimental cultures divided by mean cpm in control cultures. SI equal or more than 2 indicates positive response.



RESULTS

Table 4: *Results of SEM, BeLPT and MeLPT performed in all patients*

	Diagnosis	Metals tested by SEM	Metals tested by Melisa	MeLPT	BeLPT	
1	SA; NCG(-)	Al, Cu, Si	Al, Cr, Si, Ti, Hg	Ti(+)	Be(-)	Copper industry
2	SA; NCG (+)	Fe, Ti, Al, Si	Al, Si, Ti	(-)	Be(-)	Military Industry
3	SA; NCG (+)	Si, Cr, Ni, Fe	Cr, Fe, Ni, Si, Ti, Be	Ti(+)	Be(-)	Metal Worker
4	SA; NCG (+)	Si, Cr, Ni, Fe, Mo	Cr, Au, Hg, Pa, Ti, Ni, Mo	Ti(+) Pa(+)	Be(-)	Jewelry industry
5	SA; NCG (+)	Au, Zn, Si, Al,	Al, Au, Hg, Pa, Si, Ti, Zn, Be	Hg(+)	Be(-)	Aircraft Industry
6	SA; NCG (-)	Al, Ag, Cu	Ag, Cu, Al	(-)	Be(-)	Welder
7	SA; NCG (+)	Al, Si, Fe, Zn	Hg, Ni, Si, Ti, Zn, Pb, Ti	Ti(+) Pb(+)	Be(-)	Welder
8	SA; NCG (+)	Al, Si Fe	Al, Au, Fe, Si, Ti, Be	Ti(+) Be(+)	Be(-)	Military Industry
9	CBD NCG (+)	(-)	Fe, Ni, Ti, Be	(-)	Be(+)	Dental technician
10	CBD NCG (+)	Al, Si Cr, Fe, Ni	Al, Cr, Fe, Ni, Si, Be	Si(+)Fe (+)Cr(+) Ni(+) Be(+)	Be(+)	Dental technician
11	SA; NCG (+)	Si, Cr, Co, Fe, Ti, Ni, W, Mn	Cr, Co, Fe, Mn, Ni, Si, Ti,	(-)	Be(-)	Dental technician
12	SA; NCG (+)	Fe, Cr, Ni, Si	Cr, Hg, Fe, Ni, Si, Ag, Sn	Si(+)Cr(+) Ni(+)	Be(-)	Teacher
13	SA; NCG (+)	Si, Au, Cr, Fe	Au, Hg, Ni, Pa, Pt, Si	Ni(+)	ND	Silicone Implants
14	SA; NCG (+)	Si	Au, Ni, Pa, Pt, Si	Ni(+)	ND	Silicone Implants

SA=Sarcoidosis; NCG= Non Caseating Granulomas; Al: Aluminum; Cu= Copper; Si=Silica; Fe=Iron; Ti= Titanium; Cr= Chrome; Ni=Nickel; Au; Gold; Mo=Molybdenum; Zn= Zinc; Hg=mercury; Mn=Manage; Co=Cobalt; Pb=Lead; W=Tungsten; Pa: Palladium

- Metal selection for MeLPT was done according to SEM results and exposure history.
- In 10/14 sarcoid like patients metal sensitization was identified by MeLPT - 13/14 of them showing non caseating granulomas.
- In 2/14 sarcoid like patients were clinically diagnosed with no histological evidence of non caseating granulomas.
- Concordance for BeLPT results was confirmed in 1/2 CBD

CONCLUSIONS

1. SEM analysis is the first step in the evaluation of sarcoid like metal exposed patients in order to demonstrate exposure.
2. The metals identified by SEM are not always those with immunogenic properties.
3. Melisa® lymphocyte proliferation test is the second step to identify metal sensitization in sarcoid like metal exposed patients.
4. The metals tested are chosen according the occupational anamnesis and questionnaire.
5. The role of lymphocyte proliferation tests[4] in assessing occupational sensitization and disease is essential in identification of metals that cause " sarcoidosis"

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