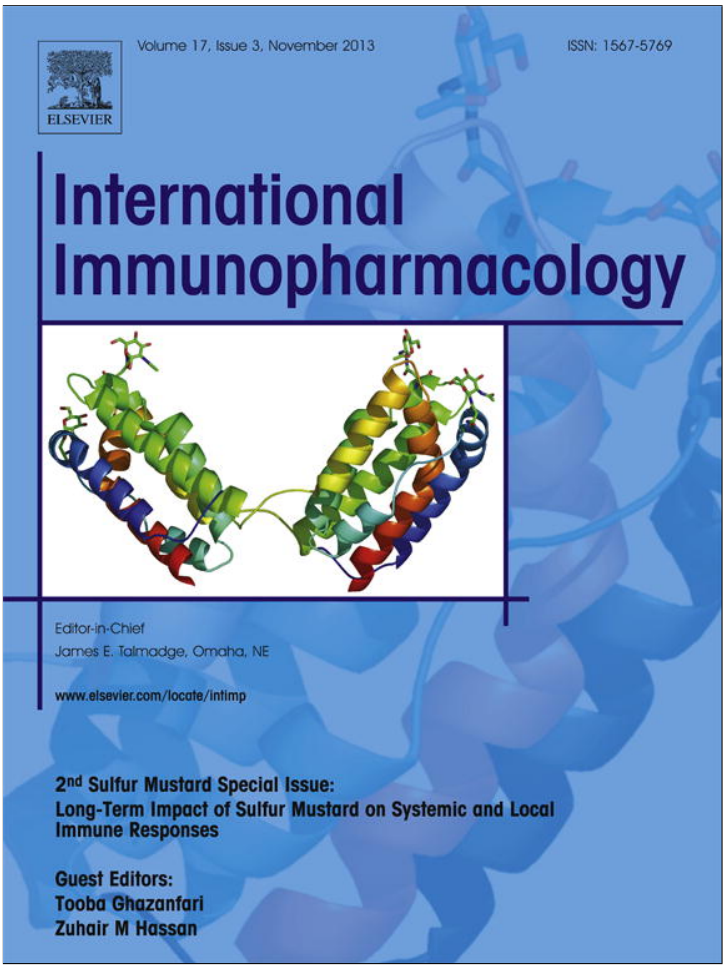

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Long term impact of sulfur mustard exposure on peripheral blood mononuclear subpopulations — Sardasht-Iran Cohort Study (SICS)

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ABSTRACT

The most important long-term morbidity problem of sulfur mustard (SM) toxicity is pulmonary complications but the pathogenesis of these complications is not clearly understood. This study evaluates the peripheral blood mononuclear sub-sets and their correlation with pulmonary function in SM exposed civilian cases 20 years post-exposure as gathered in the context of the Sardasht-Iran Cohort Study (SICS). Samples were randomly selected from two groups, SM-exposed (n = 372) and control (n = 128), with the same ethnicity, culture, and demography. Three color flow cytometry was applied for peripheral blood mononuclear sub-population determination. Results indicated a significant decrease in CD45 + /CD3 +, CD45 + /CD3 + /CD4 +, and an increase in CD3 + /CD16 + 56 + percentages. It was also found that absolute count of NK cells was highly increased in peripheral blood of exposed cases. There was a significant increase in NK cell count of SM exposed group with pulmonary problems as compared to the same group without pulmonary problems (p-value < 0.04) based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD). The findings showed a significant negative correlation between absolute numbers of T lymphocyte and FVC % and positive correlation with FEV1/FVC%. The results also demonstrated that absolute numbers of monocytes had a negative correlation with FVC %. We propose that NK and T cells are probably involved in the pathogenesis or immune reactions to the delayed pulmonary complications induced by SM. This hypothesis should be tested in a more severe pulmonary complicated group.

1. Introduction

The most important long-term morbidity problem of Sulfur Mustard (SM) toxicity is pulmonary complications but the pathogenesis of these complications is not clearly understood. As has been reported, SM exposure can lead to the development of a series of chronic destructive pulmonary complications. Different diagnoses including asthma, chronic bronchitis, bronchiectasis, airway narrowing due to searing or granulation tissue, pulmonary fibrosis [1] obliterative bronchiolitis (OB) [2,3], bronchiolectasis, and chronic obstructive pulmonary disease (COPD) [4] were reported but there are unknown cell and molecular mechanisms underlying SM induced lung complications. It is well

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known that SM exposure has considerable impact on hematopoiesis and peripheral blood sub-sets. Few reports on long term effects of SM on leukocytes sub-sets have been published previously. It was shown that CD4 and CD8 T cells were decreased in SM exposed veterans long after exposure [5–7]. However, Mahmoudi et al. showed an increase in CD3 + and monocytes in SM exposed cases [8].

A significant depression has been reported on NK cells in veterans who had severe SM-related complications 16 years after exposure [8]. Low NK cell percentage was also reported in another study in severe SM exposed patients, however an elevation was reported in their NK activity [9].

To investigate long term complications of SM exposure and the underlying mechanism(s) a historical cohort study has been established, named 'Sardasht-Iran Cohort Study' (SICS) [10].

In this paper the results of peripheral blood mononuclear subsets and their correlations with SM induced pulmonary findings are reported.

2. Materials and methods

2.1. Participants

The details of methodology of SICS was extensively described in the original methodology paper [10]. In SICS, 372 male participants from Sardasht, who were exposed to SM in 1987, were compared to 128 unexposed sex/age matched controls from the unexposed town of Rabat. SICS was initiated in 2006. The clinical evaluations and sample preparations were undertaken in 2007.

2.2. Ethical considerations

The study was approved by the Ethical committee of Board of Research Ethics of Janbazan Medical and Engineering Research Center (JMERC), the Board of Research of Ministry of Health, and Shahed University. Those recruited were individuals who voluntarily participated in the study after signing the informed consent form.

2.3. Clinical evaluation

A questionnaire containing pulmonary symptoms (chronic cough, sputum, hemoptysis, and dyspnea) and pulmonary findings (crackles, rales, and wheezing) was completed by internists and the patients were examined at the same time. Chronic cough was defined as coughing for more than 3 weeks. Spirometry was also performed for all participants, according to American Thoracic Society Criteria with spirometry device (Chest 801 Spirometry) in three consecutive measurements under the supervision of a well-experienced nurse and the best measurement was selected. The classification of severity of pulmonary involvement was done according to the Global Initiative for chronic Obstructive Lung Disease (GOLD). There was no one in grade IV severity according to GOLD classification. In addition, the cases were classified as normal, mild, moderate or severe based on the criteria verified by the Iranian Medical Committee of the Foundation of Martyrs and Veterans Affairs [10].

2.4. Blood sampling and flow cytometry

Peripheral blood samples were drawn into EDTA-treated vacutainer tubes (BD Biosciences) for immuno-phenotyping. All the samples were processed within 2 h after blood collection.

Peripheral blood leukocyte populations were studied using three color staining reagents. Staining reagents purchased from BD Biosciences (Erembodegem, Belgium) included CD45/CD3/CD4 (cat#340383), CD45/CD3/CD8(cat#340344), CD45/CD14(cat#340040), CD45/CD3/CD19(cat#340381), CD45/CD3/CD16CD56(cat#340300), CD3/HLA-DR(cat#340048) and CD3/CD4/CD25(cat#341134). Manufacturer's instructions were followed for sample processing. 50 μl of EDTA-treated blood was dispensed into each tube already containing fluorescence conjugated antibodies, incubated for 10 min at room temperature in the dark, washed twice with 2% FBS/PBS, fixed with FACS Fix buffer (BD Biosciences), washed twice again and re-suspended in wash buffer. The cells were then read within 24 h after processing. The cells were gated based on SSC and CD45 +. Subsequently, the percentage of each population was determined. Appropriate isotype controls were used to set the quadrants. The samples were read using FACS Calibur and analyzed using FlowJo (TreeStar). In addition, WBC count was determined using automated blood cell counters in order to calculate absolute number of each cell population.

2.5. Statistical analysis

Demographic data was presented using frequencies (percentages) and the comparison between control and exposed group was determined by Chi-square test. The results of hematological tests and flow cytometry were presented as mean ± standard deviation and were compared between the study groups by *t*-test. The Chi square test was used to compare pulmonary symptoms and auscultation between exposed and control groups; the difference in PFT values between the two groups was evaluated by *t* test. The CD markers were compared between the pulmonary problematic participants and participants without pulmonary problems within each study group and between exposed and control group with Mann–Whitney. The analysis was done with SPSS 16 (SPSS Inc., Chicago, Illinois) and *P* value less than 0.05 was considered as the significant level.

3. Results

There were no significant differences between the two groups in terms of age, Body Mass Index (kg/m^2), marital status and smoking. The mean ± SD of the age in the control and SM exposed group were 41.7 ± 9.8and 43.8 ± 10.8 respectively.

3.1. Hematologic result

Hematology data showed a significant reduction in WBC (7375.7 ± 1963.3 vs 7954.3 ± 2199.8 $10^3/\mu l$, p = 0.006) and in the PMN (polymorphonuclear) cell percentage (60.88 ± 6.19 vs 63.54 ± 6.44, p = 0.001) in the exposed group. Conversely, the lymphocyte percentage was increased in the exposed group (35.07 ± 6.11) compared to the control (32.30 ± 6.44) (Table 1) [11].

3.2. Flow cytometry results

Flow cytometry data showed a significant increase in the percentage and absolute number of NK cells in the exposed (9.67 ± 4.97% and 239.21 ± 124.95 count) compared to the control (7.07 ± 3.71% and 179.90 ± 111.21 count) with p = 0.001 and p = 0.0001 for percentage

Table 1
Comparison of hematological finding between study groups.

	Control N = 128 mean ± SD	Exposed N = 367 mean ± SD	p-Value
WBC ($10^3/\mu l$)	7954.3 ± 2199.8	7375.7 ± 1963.3	0.006
PMN (%)	63.54 ± 6.44	60.88 ± 6.19	<0.001
Lymphocyte (%)	32.30 ± 6.44	35.07 ± 6.11	<0.001
Monocyte (%)	3.03 ± 1.16	2.85 ± 1.19	0.138
Eosinophil (%)	1.09 ± 1.22	1.13 ± 1.53	0.758
Basophile (%)	0.02 ± 0.12	0.03 ± 0.19	0.428

WBC count was determined using automated blood cell counters in order to calculate absolute number of each cell population for SM exposed and the control groups. As seen in the table there is significant reduction in white blood (WBC) count and % of polymorphonuclear cells (PMN) however lymphocyte percentage showed significant increase in SM exposed cases compared to the controls.
(This table was previously published by Shams et al., in Toxin Reviews, 2009, Ref [11]).

and absolute number respectively (Table 2). The percentage of CD3+ and CD4+ cells showed a statistically significant decrease in exposed group. However the percentage of other leukocytes (CD45+) sub-sets including CD3+/CD8+, CD3+HLA-DR+, CD19+ and CD14+ did not show any significant difference. The presence of CD25 was also evaluated as a marker for activated T lymphocytes, but no significant alteration was found in the percentage and absolute number of CD3+/CD4+/CD25+ cells between control and exposed groups.

Correlation analysis was performed between lymphocyte and NK cell counts and significant correlation was demonstrated in both control ($r = 0.527$, $p < 0.0001$) and exposed ($r = 0.382$, $p < 0.0001$) groups (Table 3).

3.3. Pulmonary findings and correlations with leukocyte sub-populations

There was a significant increase in NK cell count of SM exposed cases who had problem in pulmonary function test compared to SM exposed cases who did not have problem in pulmonary function test based on GOLD, p-value = 0.036 (Table 4).

There was a significant increase in NK cell count of SM exposed cases who had pulmonary problem (0.006) and who did not have pulmonary problem (<0.001) based on the criteria verified by the Iranian Medical Committee of the Foundation of Martyrs and Veterans Affairs, compared to parallel controls (Table 5). However, there was no significant increase in NK cell number in SM exposed patients with and without pulmonary problem.

The Spearman correlation coefficient between CD markers and Spirometry findings showed that absolute numbers of T lymphocyte has a significant negative correlation with forced vital capacity (FVC) % and positive correlation with forced expiratory volume in 1 s (FEV1)/FVC%. There was also a significant positive correlation between B and activated T cells with peak expiratory flow (PEF) %. The results also showed that absolute numbers of monocytes had a significant negative correlation with FVC % (Table 6).

There was no correlation between absolute number of leukocyte sub-populations with sign or symptoms in the study groups (data not shown).

4. Discussion

The pathology and the immunological mechanisms underlying pulmonary complications caused by SM are not clearly understood, however different pathological diagnoses have been reported including COPD [4], bronchiolectasis, oblitrative bronchiolitis [3] and pulmonary fibrosis [1].

The roles of different leukocyte sub-sets in lung inflammatory diseases have been demonstrated. In SM injuries there are controversial reports regarding the proportion of leukocyte sub-sets.

Hematological results showed a decrease in total WBC and PMN cells but an increase in lymphocyte numbers in the exposed group. There are discrepancies among previous reports regarding hematological results in SM exposed cases [3,6,12]. This may be due to the sample selection methods, samples size, and the duration of exposure.

Our results did not show any difference in CD3, CD4, CD8, CD19, and CD14 absolute numbers however the percentage of CD3 and CD4 were decreased. Previous studies have shown conflicting results, with both increases and decreases in peripheral blood CD4 and CD8 percentage reported in long-term post-exposure SM cases [5,7,12]. However, these studies only reported the percentages of different leukocyte sub-sets. In the present study, three colors staining with CD45 leukocyte gating and absolute subset count calculation has been performed, which is more reliable than percentages.

The discrepancies could also be attributed to: 1) ethnic divergence in participants of the other studies, 2) sample size, 3) age range (which is 20–60 in our study), 4) severity of problems, 5) intensity, frequency, and duration of exposure, and 6) history of therapy.

Table 3
Spearman's rho correlations between lymphocyte count with NKC, T- and B-cells.

		Lymphocyte count	
		Control	Exposed
CD3-CD16/56+ count (T cells)	r	0.527	0.384
	p	<0.001	<0.001
	N	111	285
CD45+/CD3+/CD19-	r	0.909	0.882
	p	<0.001	<0.001
	N	111	284
CD45+/CD3-/CD19+ (B cells)	r	0.675	0.660
	p	<0.001	<0.001
	N	111	285

Spearman's rho correlations between lymphocyte count and NKC (CD3-CD16/56+), T cell (CD45+/CD3+/CD19-) and B cell (CD45+/CD3-/CD19+) in SM exposed and the control groups. As seen in the table there is statistically significant direct correlation between NKC, T and B with lymphocyte count in both groups of study.
r: Spearman's rank correlations coefficient. p: p-value two tailed test, N: number.

Table 2
Comparison of lymphocyte sub-populations (percentage and absolute numbers) in study groups.

	Percentage			Absolute numbers		
	Control (N = 111) mean ± SD	Exposed (N = 284) mean ± SD	p-Value	Control (N = 111) mean ± SD	Exposed (N = 284) Mean ± SD	p-Value
CD45+/CD3+/CD19- (T cells)	62.29 ± 7.63	59.41 ± 8.57	**0.002**	1559.1 ± 464.4	1515.7 ± 505.0	0.433
CD45+/CD3-/CD19+ (B cells)	12.30 ± 3.65	13.15 ± 4.45	0.074.	309.9 ± 136.3	337.0 ± 160.9	0.118
CD45+/CD3+/CD4+	39.01 ± 6.57	36.59 ± 7.67	**0.004**	976.9 ± 310.9	935.1 ± 347.4	0.269
CD45+/CD3+/CD8+	23.64 ± 6.55	22.31 ± 6.23	0.060	591.7 ± 226.5	569.5 ± 245.4	0.410
CD45+/CD3-/CD16+/CD56+ (NK cells)	7.07 ± 3.71	9.67 ± 4.97	<**0.001**	179.7 ± 111.2	239.2 ± 124.9	<**0.001**
CD45+/CD14+ (monocytes)	4.95 ± 1.84	4.86 ± 2.24	0.706	392.8 ± 177.4	358.4 ± 211.0	0.129
CD3+/CD4+/CD25+	14.57 ± 4.76	14.14 ± 5.41	0.465	366.2 ± 163.9	357.7 ± 171.6	0.655
CD45+/CD3+/HLA-DR+	11.57 ± 4.66	10.85 ± 5.12	0.201	289.4 ± 144.0	277.9 ± 161.6	0.513

Absolute number and percentage of lymphocyte sub-populations in peripheral blood of SM exposed and control groups were assessed using three color staining reagents. Staining reagent groups purchased from BD Biosciences (Erembodegem, Belgium) included CD45+/CD3+/CD4+, CD45+/CD3+/CD8+, CD45+/CD14+, CD45+/CD3-/CD19+, CD45+/CD3+/CD16+56+, CD45+/CD3+/HLA-DR+ and CD3+/CD4+/CD25+. Manufacturer's instructions were followed for sample processing. Lymphocytes were gated among all CD45+ cells. Appropriate isotype controls were used to set the quadrants. The samples were read using FACS Calibur and analyzed using Cellquest software (BD Biosciences). P value: Comparison of study groups (*t* test).
Bold data shows significant differences with p value <0.05.

Table 4
CD45+/CD16/56+ (count) in control and exposed groups with Pulmonary Function (GOLD).

Study groups	Pulmonary function (GOLD)	CD45+/CD16/56+ (count)						p-value[1]	p-value[2]
		N	Median	Q1	Q3	Mean	SD		
Control	Without problem	96	150.1	101.0	225.5	177.1	111.0	0.336	**0.000**
	Problematic	13	211.5	126.2	284.5	208.3	118.1		0.148
Exposed	Without problem	234	216.7	148.9	282.8	231.0	115.6	**0.036**	
	Problematic	43	243.1	175.6	388.8	292.7	159.1		

The absolute number (count) of CD45+/CD16/56+ in control and exposed groups who had and who did not have pulmonary problems were presented as Median with First (Q1) and third (Q3) quartile and also mean ± SD.
Problematic: Those participants who had pulmonary problem (stages I, II and III based on GOLD classification).
Without problem: Those participants who had normal condition based on GOLD classification.
There was no one in grade IV severity according to GOLD classification and only the grades from I to III were reported.
p-value[1]: comparisons of CD45+/CD16/56+ (Count) between participants without pulmonary problem and Problematic participants within each study group (Mann–Whitney).
p-value[2]: comparisons of CD45+/CD16/56+ (Count) between study groups (Mann–Whitney).
Bold data shows significant differences with p value <0.05.

Most likely, though, the absolute number of NK cells has caused the increase in lymphocyte numbers. The absolute number of NK cells in the exposed group was significantly higher than in the control group and a significant positive correlation was demonstrated between lymphocyte count and NK cells count in both groups (Table 4). The impact of NK cells on tissue remodeling and degeneration was recently studied in chronic obstructive pulmonary disease (COPD) [13]. It has been demonstrated that peripheral NK cells of COPD smokers are less cytotoxic than those of healthy smokers. In addition, the number of NK cells in both the peripheral blood and broncho-alveolar lavage (BAL) is decreased in COPD. Notably, another killer subset, NKT cells, increased both locally and peripherally in COPD [13]. The increase of NK cell numbers in the peripheral blood of the SICS is in contrast to COPD reports implying the involvement of different pathological mechanisms. Further investigation must be done to elucidate NK activity by determining cytokine production, granzyme/perforin content in addition to phenotypic analysis for NKT [14]. In many research works and some textbooks it has been mentioned that T lymphocyte and NK cell numbers may compensate for one another this matter may be relevant for our findings [15].

Our results are not in agreement with other reports of veteran cases 16 years after exposure [8]. It is noteworthy that using three color staining in SICS study provided more reliable results than two color staining in previous report. A study on BAL of selected group of SM exposed with pulmonary fibrosis (PF) showed no significant difference in NK either percentage or absolute number compared to the control group. In addition, they showed an increase in CD8+ T cells along with decrease of CD4+ T cells in the BAL of SM exposed cases with PF. In that study a particular pathology feature was selected and the results were compared to the unexposed cases without PF. Since the inclusion criteria in that study differs from the present study, we cannot compare the results to show the impact of leukocytes sub-sets on pulmonary complications in SM exposed cases.

The results of the present study showed that NK cells were increased in SM exposed with pulmonary problem based on GOLD classification compared to cases without pulmonary problem (Table 4). However, if the criteria verified by the Iranian Medical Committee of the Foundation of Martyr and Veterans Affairs for evaluation of pulmonary problems have been used, this difference does not exist (Table 5). One explanation is the different parameters used in these criteria.

There is no comparable study to show the impact of NK cells in SM induced lung damage. In lung transplantation with BO the proportion of peripheral blood NK cells were elevated compared to the stable patients without BO [16]. Ghanei et al. have reported bronchiolitis obliterans (BO) as the main late respiratory complication of SM exposure [2], our findings regarding elevated numbers of peripheral blood NK cells confirm the Ghanei et al. findings. Recently COPD was also reported as a late toxic pulmonary consequence after SM exposure by Ghanei et al. [4]. However, they indicated that the COPD observed in these patients is unique (described as Mustard Lung) and to some extent different from COPD resulted from other well-known causes. In a study on the pathogenesis of COPD due to smoking it has been shown that the proportion and activity of peripheral blood NK cells has decreased compared to healthy smokers and non-smokers [13]. Since in the present study NK cell numbers were increased in SM exposed group we proposed that a unique patho-physiological mechanism might be involved in pulmonary complications induced by SM. It is also reported that patients with IPF showed a higher percentage and absolute number of NK cells in peripheral blood [17,18]. Pulmonary fibrosis is the other clinical diagnosis in some SM exposed patients which is previously reported by Emad [19]. Elevated numbers of NK cells in peripheral blood is in agreement to this diagnosis but in IPF patients a Th2 cytokine profile is involved in pulmonary complications whereas in our study (SICS) Th2 is decreased and a dominant Th1 response is shown (submitted manuscript). Although the role of NK cells in lung diseases is not fully understood, it is proposed by some researchers that NK cells may control the balance between health and pathology in the lung, and thus understanding their actions may help to identify novel targets for immunomodulation in respiratory disease [20].

Table 5
CD45+/CD16/56+ (count) in control and exposed groups with pulmonary assessment.

Study groups	Pulmonary assessment	CD45+/CD16/56+ (count)						p-value[1]	p-value[2]
		N	Median	Q1	Q3	Mean	SD		
Control	Without problems	72	142.96	107.14	225.51	179.49	114.31	0.837	<0.001
	With problems	35	154.27	83.10	253.59	175.58	110.10		0.006
Exposed	Without problems	160	217.73	149.15	287.25	237.59	122.59	0.827	
	With problems	116	223.83	155.35	294.33	243.01	128.83		

The absolute number (count) of CD45+/CD16/56+ in control and exposed groups at different severity stages of pulmonary assessment were presented as median with first (Q1) and third (Q3) quartile and also mean ± SD.
Pulmonary assessment: The classification of severity of pulmonary involvement in SM exposed patients according to clinical assessment using criteria verified by the Iranian Medical Committee of the Foundation of Martyr and Veterans Affairs.
p-value[1]: Comparison of participants who had mild, moderate, or sever pulmonary problems with participants who did not have pulmonary problem within each study group (Mann–Whitney), p-value[2]: Comparison of parallel control and exposed group (with or without pulmonary problems)(Mann–Whitney).

Table 6
Correlation between lymphocyte sub-sets and spirometry parameters in SM exposed and control group.

		FVC %		FEV1%		FEV1/FVC%		MMEF%		PEF%	
		Control	Expose	Control	Expose	Control	Expose	Control	Expose	Control	Expose
CD45 +/CD3 + (count)	r	0.103	−0.141	−0.063	−0.084	0.049	0.159	−0.040	−0.051	−0.131	−0.071
	p	0.288	**0.019**	0.515	0.163	0.709	**0.040**	0.764	0.560	0.177	0.278
CD45 +/CD19 + (count)	r	0.002	0.007	−0.121	0.052	−0.198	0.106	−0.190	0.129	−0.077	0.129
	p	0.980	0.903	0.210	0.386	0.130	0.171	0.145	0.140	0.431	**0.046**
CD45 +/CD3 +/CD4 + (count)	r	0.116	−0.105	−0.058	−0.076	0.027	0.107	−0.029	−0.072	−0.123	−0.057
	p	0.229	0.082	0.550	0.209	0.836	0.165	0.826	0.412	0.206	0.380
CD45 +/CD3 +/CD8 + (count)	r	0.036	−0.089	−0.091	−0.075	0.082	0.002	−0.011	−0.147	−0.121	−0.080
	p	0.707	0.141	0.345	0.215	0.532	0.982	0.935	0.092	0.214	0.217
CD45 +/CD16/56 + (count)	r	0.104	−0.064	0.002	−0.056	−0.021	0.048	−0.103	−0.073	−0.044	0.026
	p	0.283	0.292	0.980	0.352	0.876	0.533	0.436	0.406	0.652	0.686
CD3 +/CD4 +/CD25 + (count)	r	−0.070	−0.064	−0.175	−0.077	−0.106	−0.029	−0.227	−0.142	−0.142	0.027
	p	0.469	0.287	0.069	0.204	0.422	0.708	0.081	0.104	0.143	0.683
CD3 +/HLA-DR + (count)	r	0.076	−0.042	−0.108	−0.005	−0.014	0.077	−0.163	0.080	−0.004	0.150
	p	0.430	0.482	0.264	0.930	0.913	0.318	0.214	0.363	0.968	**0.020**
CD45 +/CD14 + (count)	r	0.021	−0.119	−0.108	−0.117	−0.195	−0.010	−0.213	−0.046	−0.038	−0.098
	p	0.825	**0.048**	0.265	**0.052**	0.136	0.902	0.103	0.600	0.700	0.131

The absolute number of leukocyte sub-sets and the pulmonary function parameters (FVC, FEV1, FEV1/FVC, MMEF, and PEF) were assessed. The correlation between the absolute number of leukocyte sub-sets and pulmonary function parameters was undertaken in the SM exposed and control groups.
r: Spearman's correlation coefficient, p: p-value, FVC: forced vital capacity, FEV1: forced expiratory volume in 1 s, MMEF: maximum midexpiratory flow, PEF: peak expiratory flow. Bold data shows significant differences with p value <0.05.

The results showed that absolute numbers of T lymphocyte has a little but significant negative correlation with FVC % and positive correlation with FEV1/FVC%. This finding confirms a role for T lymphocyte in SM induced late pulmonary complications. There was also a significant positive correlation between B and activated T cells with PEF %, and also absolute numbers of monocytes had a little, but significant negative correlation with FVC % (Table 6). At this time we cannot precisely interpret the results as we do not analyze any biopsies however the cellular composition of sputum is under investigation. Further investigations are being undertaken to elucidate pathophysiological meaning of those findings.

In this study CD3 + T cells and CD3+CD4 + T cells percentages were decreased, but their absolute number did change, this reduction in T cell percentages may be due to increased number of NK cells this is confirmed by a statistically significant direct correlation between NK cells and lymphocyte count in both groups of study.

In conclusion, this study implies that NK and T cells have a role in delayed pulmonary complications induced by SM. Additional research on their function in addition to local evaluation in lungs of severe patients is necessary for better understanding of the cellular events.

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