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CHANGES IN THE T-LYMPHOCYTES (CD3+CD19-) AND ACTIVATED T-LYMPHOCYTES (CD3+HLA-DR+) IN PATIENTS OVER 60 WITH MEDITERRANEAN SPOTTED FEVER

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ABSTRACT

Mediterranean spotted fever (MSF) in Bulgaria shows increasing severity, especially in patients over sixty years. As T lymphocytes are the primary effector cells in MSF and are altered by the ageing process we aimed to specify the changes in their numbers in elderly patients. We performed a clinicoepidemiological study, haematological and biochemical analysis of 132 patients of 60+ and 30 patients between 19 and 57 years with MSF. We investigated the cell immunity of 20 patients of 60+ using immunofluocytometry. The control group consisted of 10 younger patients and 10 healthy individuals of 60+. MSF showed more unfavourable course in the elderly.We found T-lymphocytes depletion in all patients with MSF. Activated T lymphocytes were increased in elderly patients with MSF. We found significant differences between the number of activated T lymphocytes in elderly patients and healthy elderly persons. The observed T-lymphocytes depletion in MSF corresponded to the disease severity. The increase in the activated T lymphocytes in patients over 60 years contributes to an adequate immune response.

UDC CODE & KEYWORDS

■ UDC: 616.9 ■ Elderly ■ Cell Immunity ■ Rickettsiosis ■ Severity.

INTRODUCTION

Mediterranean spotted fever (MSF) is the most frequent tick-borne rickettsiosis in Bulgaria in the last few years. It appeared in Varna region in 1994. Since then more than 1700 people fell ill, of which over 993 were hospitalized in an Infectious department. Nowadays, increase of the severe cases of MSF and the complications due to it are observed worldwide. These peculiarities in the clinical course of that rickettsiosis are most typical for the patients over 60 years. They represent 30, 17% of all patients with MSF that we observed. Old age is the most widely distributed type of immunodeficiency. With the ageing process the immune system begins to lose some of its functions and becomes unable to react fast and efficiently to stimulation (Whitman 1999). Alterations in the immune system due to ageing are detectable at all levels. T-cells` functions are affected in different ways (Meyer 1994). Spotted fever group rickettsia stimulates both cell mediated and humoral immunity, but T lymphocytes are the primary effector cells of the immune response (Valbuena et al. 2002). For that reason we investigated the cell immunity of 20 patients over the age of 60 with Rickettsia conori infection.

Objectives

The objectives were to specify the changes in the T- lymphocytes (CD3+CD19-) and the activated T- lymphocytes (CD3+HLA-DR+) in patients over 60 with Mediterranean spotted fever.

Methods

We investigated a total of 132 patients in the age between 60 and 85 years (median age of 71, 04±1, 77 years) with MSF, hospitalized for the period 2000-2007. Thirty patients between 19 and 57 years (median age of 42, 77±5, 86 years) served as a control group. We implemented a clinicoepidemiological study. The criteria for severity were consistent with ESCAR/ ESCMID (Brouqui et al. 2004).

We measured the levels of hemoglobin, the erythrocyte count, the white blood cells count and their differentiation, the erythrocyte sedimentation rate, the CRP levels, the platelets count, the coagulation factors, the liver enzymes, kidney function values and urine. The diagnosis was serologically confirmed using immunofluorescent assay.

We investigated the cell immunity of 20 patients of age over 60 years with MSF, using immunofluocytometry. The control group consisted of 10 young patients with MSF under 60 years and 10 healthy individuals over the age of sixty. The results were automatically analysed by SimulSet softwear (Verity Softwear House, Inc.), minimum of 50000 cells were analysed.

We used variation, alternative, nonparametric, correlative and graphic analysis during the statistical processing.

Results and discussion

Clinical characteristiscs. According to our own observations of the hospitalized patients and using adapted criteria from other authors, we formed 3 clinical groups with different severity of the course (Gospodinova 2003; Raoult 1986). The major clinical symptoms of the observed patients are shown in Table 1. Decreased adaptive abilities, changed homeostatic mechanisms and comorbidity make people of 60+ extremely susceptible to the infection with R.conorii. In 79, 54% (105) of the patients of 60+ chronic conditions were present: cardiovascular disease, cerebrovascular disease, chronic obstructive pulmonary disease, pneumopathia, diabetes mellitus, renal disease.

Fifty seven (43, 18%) of the elderly patients presented severe form of the disease, 61(46, 21%) - moderate form, 14 (10, 61%) - mild form.

From the 132 elderly patients studied we observed mental changes in 13 of them, 3 were in coma during the first few days. In the same group, 3 patients had focal neurological symptoms (pareses and seizures) and 4 patients presented with signs of meningeal irritation. The above conditions were estimated as a result of the development of cerebral edema with meningismus, and in one case - as a result of stroke. Thirty one patients (23, 48%) of the group "60+" had disturbances in the renal function - 11 in a mild form, 20 in a severe form. In the control group we have noted mental changes in only 2 patients, paresis was diagnosed in 3, meningismus – in 3, during the first days. Only three of the younger ones (10%) had disturbances of the renal function in a mild form. MSF had a fatal outcome in 13 (1, 30%) of the hospitalized elderly patients. The presence of chronic diseases, delay in the beginning of appropriate etiologic treatment, the presence of malnutrition and the poor socioeconomic conditions predetermine the clinical course and outcome for the patients of 60+ with MSF.

We observed similar clinical presentation in the immunologically investigated 20 patients of 60+ in the years 2006-2007. Five patients presented with a severe form of the disease, 12 – with a moderate form and 3 – with a mild form (Table 1a).

Table 1: Major symptoms in patients over 60 years of age with MSF

Symptoms	Patients 60+	Control group	t	p
	n= 132	n=30		
		(20 – 54) years old		
Fever:				
Up to 38°	20,7%	50,0%	2,8	< 0,05
Over 39°	79,2%	50,0%	2,8	< 0,05
Up to the third day	32,4%	40,0%	0,73	> 0,05
Up to the fifth day	32,4%	40,0%	0,73	> 0,05
Up to the seventh day	22,0%	10,0%	1,66	> 0,05
Up to the 10-th day and more	12,9%	10,0%	0,44	> 0,05
Tache noire	88,3%	63,3%	2,63	< 0,05
Conjunctival and pharyngeal injection	84,4%	73,3%	1,2	> 0,05
Rash:				
Papulous	3,8%	6,7%	0,47	> 0,05
Maculopapular	77,3%	80,0%	0,57	> 0,05
Hemorrhagic	18,9%	13,3%	0,64	> 0,05
Abundant	88,3%	63,3%	2,63	< 0,05
Scanty	11,6%	36,6%	1,94	< 0,05
Leucocytosis	15,5%	20,0%	0,54	> 0,05
Low platelets count	36,3%	23,3%	1,38	> 0,05

Source: Author

Table 1a: Major clinical symptoms in the immunologically investigated patients 60+ with MSF

Symptoms	Mild clinical form, n=3(15%)	Moderate clinical form, n=12(60%)	Severe clinical form, n=5(25%)	Investigated patients, n=20
Fever:				20-100%
T - up to 38° C	3-100%	0	0	3-15%
T - 38°-38,5°C	0	7-58,33%	0	7-35%
T - over 39° C	0	5-41,67%	5-100%	10-50%
Tache noire	3-100%	12-100%	5-100%	20-100%
Conjunctival and pharyngeal injection	2-66, 7%	10-83,33%	5-100%	17-85%
Papulous	0	1-8,33%	0	1-5%
Maculopapular	3-100%	9-75%	3-60%	15-75%
Purpura	0	2-16,67%	2-40%	4-20%
Abundant rash	0	12-100%	5-100%	17-85%
Scanty rash	3-100%	0	0	3-15%
Leucocytosis	0	3-25%	2-40%	5-25%
Leucopenia	0	1-8,33%	1-20%	2-10%
Left shift	2-66,67%	7-58,33%	4-80%	13-65%
Low platelets count	0	5-41,66%	3-60%	8-40%
Fibrinogen level under 2g/l	0	2-16,67%	1-20%	3-15%

Source: Author

Immunological characteristics. We found decreased numbers of the T-lymphocytes (CD3+CD19-) in patients over sixty with MSF, as well as in the control group of younger patients with MSF. T-lymphocytes were in the referent values in the control group of healthy individuals of 60+ (Table 2). This decrease is probably due to accumulation of the T-cells in

perivascular infiltrates in the course of a rickettsial infection, according to the published data (Valbuena et al. 2002). The defensive role of the T-lymphocytes is due to interferon-γ (INF-γ) activation of the endothelial rickettsiocidal mechanisms and to the cytotoxic T-lymphocytes cytolytic mechanisms (Walker et al. 2001). We found a reverse correlative dependence between T-lymphocytes numbers and age in the three investigated groups (fig.1). A moderate reverse correlative dependence also exists between the number of CD3+T-lymphocytes and the severity of the disease (r=0, 34) in the elderly patients with MSF (fig.2).

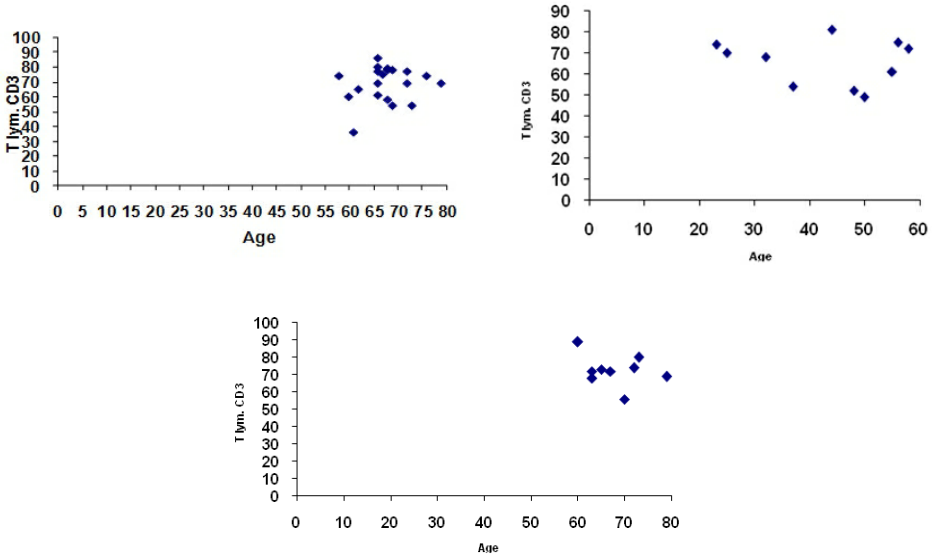
Table 2: Results of the analysis of T-lymphocytes and activated T-lymphocytes in patients with MSF and healthy elderly persons

Immunological	**MSF – 60+**	**MSF- under 60**	**Healthy persons – 60+**
indices	x±Dx	x±Dx	x±Dx
CD3+ CD19-	69,63 ± 5,46	66,42 ± 7,45	72,55 ± 5,79
CD3+HLA-DR+	27,45 ± 5,08	21,35 ± 6,52	11,35 ± 2

** x ± Δx- confidence interval; Δ - representative error*
** x – relative values in %*

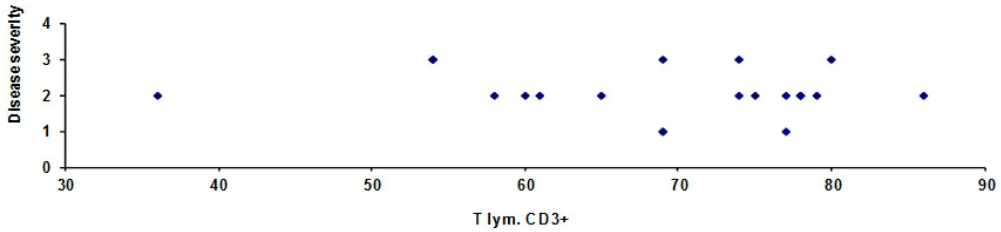
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Figure 1: Dependence between CD 3+and age in patients with MSF of 60+, under 60 years, in healthy persons of 60+



Source: Author

Figure 2: Dependence between CD 3+ and the disease severity in patients with MSF of 60 +



Source: Author

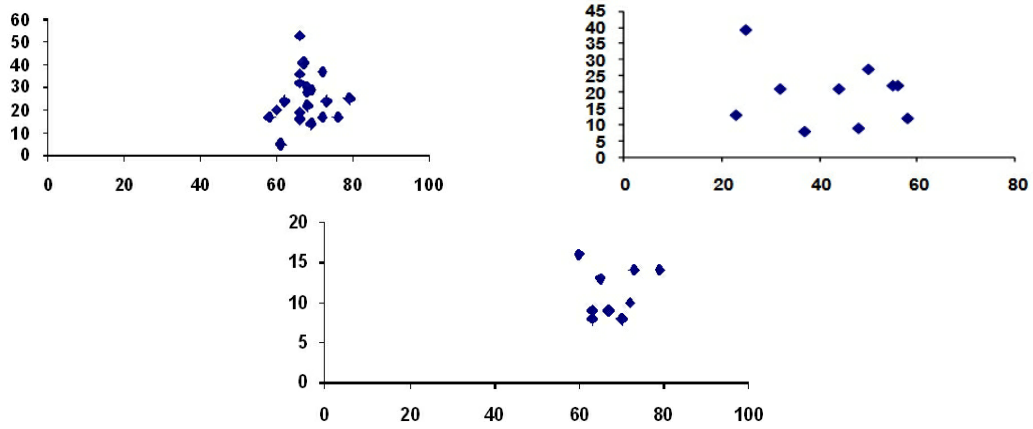
We found increased numbers of the activated T-lymphocytes (CD3+/HLA-DR+) in patients with MSF of 60+, as well as in patients with MSF under the age of 60. The activated T-lymphocytes in healthy persons of 60+ are in the referent values. These results are confirmed by the data published by other authors and correlate well with the already established pathogenesis of the rickettsioses, in particular that of the Mediterranean spotted fever (Popivanova 2003; Walker 2003). The difference between the activated T-cells in patients over 60 with MSF and those of the control group of healthy elderly people is statistically reliable, p<0,001. The observed differences between the activated T-cells in these two groups are significant and their manifestation is quite regular (t=6, 24). There is a straight dependence between the activated

T-lymphocytes (CD3+/HLA-DR+) and age in both groups - patients of 60+ with MSF and healthy elderly individuals (fig.3). In patients under 60 with MSF reverse dependence was found (fig.3).

A moderate straight dependence was established between the activated T-lymphocytes in patients under the age of 60 and the severity of the disease (fig.4).

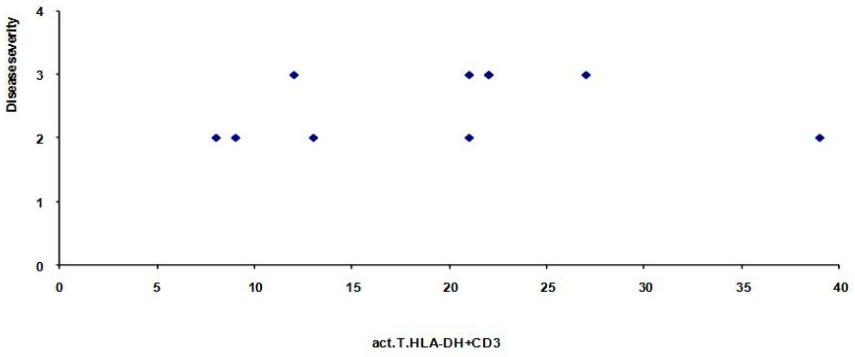
Correlative and regressive analysis of the results showed considerable dependence between the disease severity and age (r = 0, 57) in the group of patients over sixty years. In the control group of younger patients that dependence is slight (r =0, 05) (fig.5).

Figure 3: Dependence between the activated T-lymphocytes and age in patients with MSF of 60+, under 60 years and in healthy persons of 60 +



Source: Author

Figure 4: Dependence between the activated T-lymphocytes and disease severity in patients with MSF under 60 years



Source: Author

We found a slight dependence between the activated T-lymphocytes and age, i.e. the determinant is the infectious process, caused by rickettsiae. Activity of the cytotoxic T-lymphocytes develops against the infected endothelial cells and macrophages in the infectious process. That activity is likely to have a major role in the healing process. In the control group of younger patients with MSF we ascertained a weak dependency of the total T-lymphocytes and the activated T-lymphocytes of age.

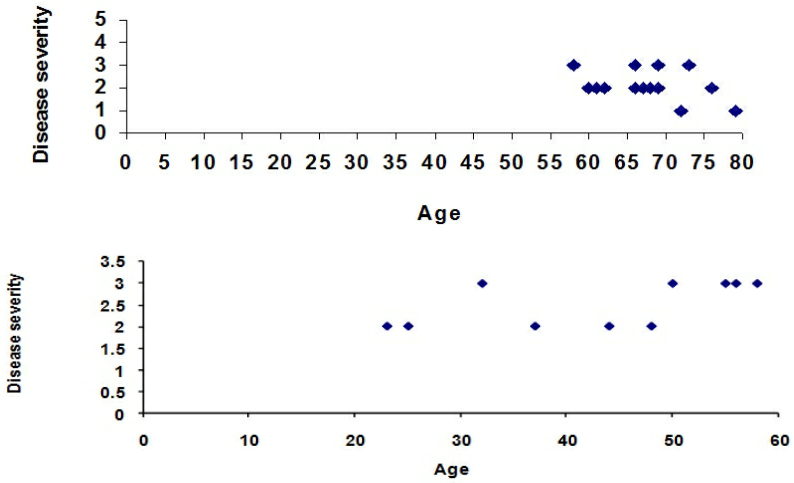
A weak dependency between both the total and activated T-lymphocytes and age was manifested in the control group of healthy individuals over 60 years.

The target cells in Mediterranean spotted fever are the endothelial cells. The proliferation of rickettsiae in the vascular endothelium results in vasculitis. The systematic vascular-endothelial rickettsial infection switches the components of the specific and nonspecific immunity. T-cells, the primary effector cells of the immune response are a major source for INF-γ release, which along with tumor necrosis factor-α(TNF-α) are necessary for the blood vessels cleaning from rickettsiae (Billings et al. 2001; Walker 2000). Immunohystological studies confirm the role of T-lymphocytes as they are found to be abundant in perivascular infiltrates in the regions of rickettsial infection (Valbuena 2002).

Multiple experiments with guinea pigs demonstrates the ability to be produced T-cell branches against recombinant OmpA and OmpB proteins, as well as strong reactivity of immune serums against them. These experiments support the significance of the cell-mediated immunity in rickettsial infections (Valbuena 2002; Diaz-Montero 2001). Activated T-lymphocytes and

NK-cells are the main defenders against rickettsial invasion. That is confirmed by several experimental data (Walker et al. 2003). The role of the infected endothelial cells as antigen presenting cells in the development of the immune reaction is also irrefutable. Our investigations confirm the leading role of T-lymphocytes in the healing process in elderly patients with rickettsiosis. Individuals over sixty exhibit adequate immune response in MSF, a fact that is strongly supported by the increase of the activated T-lymphocytes.

Figure 5: Dependence between the disease severity and age in patients with MSF of 60+, under 60 years



Source: Author

Conclusion

1. T-lymphocytes (CD3+CD19-) were found depleted in all patients with Mediterranean spotted fever, while they were in referent values in healthy individuals over 60 years. The decrease of CD3+ T-lymphocytes in patients of 60+ with MSF in comparison with the healthy persons in the same age group, corresponds to the disease severity.
2. Activated T-lymphocytes (CD3+/HLA-DR+) were found increased in elderly patients with MSF contributing to the development of an adequate immune response against the rickettsial invasion. Comparing the number of activated T-lymphocytes in elderly patients with MSF to healthy elderly persons were established reliable differences.

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