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Wide geographic variations of sensitivity to MOTT sensitins in Greece

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Wide geographic variation of sensitivity to MOTT sensitins in Greece. G.A. Dascalopoulos, S. Loukas, S.H. Constantopoulos. ©ERS Journals 1995.

ABSTRACT: Several studies have suggested that large bodies of water are a main source of infection with mycobacteria other than tuberculosis (MOTT). If this is correct, there should be a gradient in the infection rate with MOTT between mountainous and seaside areas.

To test this hypothesis, we performed skin testing with tuberculin and sensitins in 19,470 Greek Armed Forces recruits. Initially, several MOTT sensitins were used, but when it became clear that the *Mycobacterium scrofulaceum* sensitin was the most appropriate, the study was continued with it alone in 17,403 recruits. Finally, in order to evaluate the geographical distribution of sensitivity to sensitins, we studied the results of 8,507 of these recruits living in or near their birthplace. They were divided into three geophysical areas: seaside 3,389 recruits; mountains 2,692 recruits; and inland plains 2,426 recruits.

MOTT sensitivity rates were 4.1% in mountainous areas and 7.1% in seaside areas. All small Aegean islands had high MOTT rates (above 8%). In inland plains, high MOTT rates (above 8%) were observed among those living near big rivers.

This geographical distribution of MOTT sensitivity supports the theory that large bodies of water are a main source of infection with MOTT.
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Previous studies have suggested that large bodies of water, such as coastal and inland waters, may be a significant source of infection with mycobacteria other than tuberculosis (MOTT). This was first suggested by EDWARDS *et al.* [1] almost four decades ago. In their study, over 670,000 US Navy recruits were skin tested with purified protein derivative-standard (PPD-S) and PPD-B (Battey) and the highest reaction rates to PPD-B sensitin (70% or more) were found amongst residents of the Southeast States [1]. Several recent studies, again mainly from the US, have examined various environmental sources, and all indicate natural water as the primary source of human infection with MOTT [2–5].

Greece is a suitable country to test this hypothesis. It has a warm, subtropical, climate favouring MOTT [6]. It is surrounded by sea water, but its mainland, sometimes only a few kilometres from the sea, is quite mountainous. Thus, if large bodies of water are a main source of infection with MOTT, there should be a gradient between seaside and mountainous areas concerning the sensitivity to MOTT sensitins.

Sensitivity to sensitins can be used as indirect evidence of infection by MOTT, showing "...the development of hyper-sensitivity of the skin" [7]. However, since there is cross-sensitivity between *Mycobacterium tuberculosis* (MTB) and MOTT [5], a detailed procedure defining infection with MTB and MOTT was attempted in our study (see Methods).

Unlike Southeastern US and some European countries, such as Sweden [8, 9] and the Netherlands [10], where sensitivity to sensitins has been examined, Greece has still a serious tuberculosis problem [11, 12]. Therefore, bacille Calmette-Guérin (BCG) vaccination is widely applied, and we had to take this into consideration. Finally, since MOTT have only been studied once, more than 20 yrs ago in Greece [13], a preliminary phase was required to determine which sensitin was the most representative.

Population and methods

The study was conducted among Greek Armed Forces recruits, aged 19–21 yrs. Recruits with possible previous BCG vaccination (according to history, records or obvious vaccination scar) were excluded, although we and others [14–17] have shown that even successful previous BCG vaccination interferes very little with Mantoux readings.

The reactions (size of skin induration) were read after 48–72 h. The study was approved by the Scientific Ethics Committee of the Greek Armed Forces and was completed in two phases.

Phase One

Several sensitins were used to establish which was the most representative of MOTT infection. Thus, we performed

dual skin tests; Mantoux test, with tuberculin (0.1 ml PPD, 2 immunizing units (IU) Institute Pasteur) and one of the following sensitins (0.1 ml): PPD-avium, PPD-intracellulare, PPD-scrofulaceum and PPD-kansasii, (Statens Serum Institute, Copenhagen, Denmark) on 19,470 recruits from all parts of Greece. In addition, 508 recruits were tested with all five sensitins.

Previous infection with MTB or MOTT was defined as follows. Briefly, when Mantoux test was ≥10 mm, infection with MTB was assumed if the reaction to tuberculin was larger than that of the sensitin and the difference was more than 5 mm. Infection with MOTT was defined when the reaction to the sensitin was >10 mm and larger than that of tuberculin. When Mantoux was <10 mm, infection with MTB was assumed if the reaction to tuberculin was 5–9 mm and larger than that of the sensitin by at least 5 mm. Infection with MOTT was assumed when the reaction to the sensitin was >5 mm and larger than that of tuberculin. Finally, there was a subgroup where it was not possible to differentiate between MOTT and MTB infection [18–20] *i.e.* those with a reaction to tuberculin larger than to the sensitin, but <5 mm difference.

Phase Two

When it became obvious (see Results) that PPD-scrofulaceum was the most appropriate sensitin, the study was continued with this sensitin only (and tuberculin) in 17,403 recruits.

Finally, in order to study the geographical distribution, 8,896 recruits not living in, or near, their birthplace, or living in large metropolitan areas (Athens, Thessaloniki) were excluded. These two metropolises have a very mixed population of natives and inhabitants from other parts of Greece and this could create confusion. Thus, the population was limited to 8,507 recruits. These were divided into three geophysical areas (seaside 3,389 recruits; mountains 2,692 recruits; and inland plains 2,426 recruits).

The statistical analysis involved the standard Z-test for proportions of independent binomial populations and the Chi-squared (χ^2) test for homogeneity of proportions in a multinomial population [21].

Furthermore, an extension of the above mentioned Z-test, suitable for testing the equality of two proportions in a multinomial population, was derived:

$$Z=\frac{p_1-p_2}{\sqrt{2p/n}}\ ,\ p_1=\frac{x1}{n}\ ,\ p_2=\frac{x2}{n}\ ,\ p=\frac{x_1+x_2}{n}$$

employing classical statistical procedures.

Results

Phase One

In order to establish the most suitable sensitin, dual skin tests were performed on 19,470 recruits with the following results. 1) from 4,742 recruits tested with PPD-avium (and tuberculin), 319 were considered infected with MOTT (2.5%), 519 with MTB, and 183 with either or both; 2) from 4,551 recruits tested with PPD-intracellulare (and tuberculin), 88 were considered infected with MOTT (1.9%), 589 with MTB, and 124 with either or both; 3) from 5,330 recruits tested with PPD-kansasii (and tuberculin), 57 were considered infected with MOTT (1.08%), 702 with MTB, and 88 with either or both; and 4) from 4,847 recruits tested with PPD-scrofulaceum (and tuberculin), 346 were considered infected with MOTT (7.1%), 586 with MTB, and 207 with either or both.

In addition, 508 recruits were tested with all five sensitins. Ninety four recruits (18.5%) had at least one reaction >5 mm; and half of these (49 out of 94 = 51%) had the largest reaction to PPD-scrofulaceum. Respective results with the other four sensitins were as follows: 21 (22%) had the largest reaction to PPD-intracellulare, 13 (14%) to PPD-kansasii, and 12 (13%) to PPD-avium. PPD-intracellulare was positive in 38 out of 94 (40%), PPD-kansasii in 37 out of 94 (39%) and PPD-avium in 24 out of 94 (26%).

Thus, positive reactions to PPD-scrofulaceum were more common than to any other sensitin (all $Z>10.44$; $p<0.001$ for comparison in dual skin testing; and $x^2 = 190.3$; $p<0.001$ for comparison in test with all five sensitins). Since 70 of the 94 recruits with any positive skin test to a sensitin were positive to PPD-scrofulaceum (74%), one can assume that roughly 3 out of 4 previous infections with MOTT were diagnosed using PPD-scrofulaceum.

Phase Two

Including recruits from Phase One, we skin tested 17,403 recruits with PPD-scrofulaceum and tuberculin. Of these, 1,224 were considered previously infected with MOTT (7.0%), 1,523 with MTB (8.9%), and 648 (3.8%) with either or both. Had we considered only ≥10 mm skin reaction to tuberculin as indicative of previous MTB infection, the tuberculin index would have been 10.6% (1,847 of the 17,403 recruits).

As mentioned previously, for the evaluation of geographical distribution, only 8,507 of the 17,403 recruits were included; namely those living in their place of birth and outside the two major metropolises (Athens and Thessaloniki). Of 2,692 recruits living in mountainous areas, 110 (4.08%) were considered infected with MOTT and 214 (7.95 %) with MTB. Of those 3,389 recruits living in seaside areas 239 (7.05%) were considered infected with MOTT and 226 (6.66%) with MTB. The MOTT infection rate between mountains/seaside differed significantly ($Z=4.94$; $p<0.001$), whilst there was no statistically significant difference regarding infection with MTB ($Z=1.93$; $p>0.05$). It is of interest that all small Aegean islands had MOTT infection rates above 8% and low MTB infection rates (0–4%).

Finally, of 2,426 recruits living in inland plains, 195 (8%) were considered infected with MOTT and 256 (10.57%) with MTB. This was a very inhomogenous population with wide differences from area to area. A closer look at the geographical distribution revealed that most areas with high MOTT infection rates (>8%) were located around big rivers.

Discussion

The main aim of our study was to test the dominant theory of the source of infection with MOTT. These mycobacteria are commonly found in house dust, tap water, coastal waters, soil and milk [5]. The infection

is not spread by person-to-person contact [5]. Environmental reservoirs are considered to be the primary source of infection, more specifically contaminated waters. The main studies have been carried out in the Eastern United States. They have shown that the distribution of contaminated coastal and inland waters corresponds well with data from skin test sensitivity to MOTT sensitins [5].

Our study strongly supports this theory. The sensitivity to *M. scrofulaceum* sensitin was much higher among recruits born and living in the coastal areas of Greece than those living on the mountains. The phenomenon was very clear in the small Aegean islands, where the sensitivity to MOTT sensitin was highest, being in almost all islands >8%. A similar finding was observed in those recruits living in inland plains, where the subgroup living near big rivers also had relatively high rates of MOTT sensitivity (>8%). The geographical distribution of the sensitivity to tuberculin showed no preference for seaside or mountainous areas.

Several previous studies have pointed out variations in sensitin reactivity in the same country, mainly as a result of climate, distance from the sea and altitude [1, 22, 23]. In the most recent study [24] it was shown that the sensitivity to sensitins in the coastal area of Goteborg, Sweden was much higher than that of the inland rural area of Jamtland, in agreement with our findings.

Other results of this study are interesting from the Greek Public Health point of view. The most significant being the fall in tuberculin sensitivity (from 25% 20 yrs ago to 10.6% [11], with a concomitant slight increase in the sensitivity to sensitins, from 5.8 to 7.0%) [13]. This is in agreement with the universal trend in Europe [10].

In conclusion, these data and those of other studies suggest that large bodies of water are a significant source of infection with MOTT. This seems to be true whether we examine children, as in Sweden, or young adults as in Greece. It is true whether we study countries with very high tuberculosis infection rates (India) [21], very low rates (Sweden) [23], or an intermediate situation (Greece). It is true whether we study countries with tropical climate (India), subtropical climate (Greece) or arctic climate (Jamtland, Sweden).

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