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The value of tuberculin skin testing in haemodialysis patients

Anne Wauters[1], Willy E. Peetermans[2], Paul Van den Brande[3], Bart De Moor[4], Pieter Evenepoel[1], Hilde Keuleers[4], Dirk Kuypers[1], Koen Stas[4], Johan Vanwalleghem[4], Yves Vanrenterghem[1] and Bart D. Maes[1]

[1]Division of Nephrology, [2]Division of General Internal Medicine and [3]Division of Pulmonology, Department of Medicine, University Hospital Gasthuisberg, Leuven and [4]Division of Nephrology, Department of Medicine, Virga Jesse Ziekenhuis, Hasselt, Belgium

Abstract

Background. Chronic haemodialysis patients are at increased risk for developing tuberculosis (TB). Appropriate screening methods to detect latent *Mycobacterium tuberculosis* infection are required. The aim of this prospective multi-centre study was to evaluate the tuberculin skin test (TST) as a screening method for detection of *M.tuberculosis* infection in haemodialysis patients.

Methods. A total of 224 patients in two haemodialysis centres were prospectively tested, using 2 units of tuberculin PPD RT23. Up to three booster injections were given with a 7 day interval to patients not responding to the previous test. The results were compared with clinical and radiological data.

Results. The cumulative prevalence of a positive TST was 14.7% for the first test, 27.8% for the second test and 32.6% for the fourth test. There was no influence of age, gender, haemodialysis centre, dialysis efficiency, nutritional state, levels of zinc, vitamin D therapy, primary renal disease, (previous or active) immunosuppressive therapy or response to hepatitis B vaccination. There was a significant, but weak, correlation between TST positivity and a history of positive TST or TB. Chest radiography and positive TST were not correlated, yet a positive chest X-ray increased the detection of patients with latent *M.tuberculosis* infection up to 47.8%.

Conclusions. In haemodialysis patients, a positive response of $>30\%$ to repeated TST was obtained. Two consecutive TSTs were sufficient to recruit most of the booster reactions. Since only a weak correlation was found with anamnestic data, regular TST evaluation in combination with a chest X-ray, is a useful tool to detect infection with *M.tuberculosis* in haemodialysis patients.

Correspondence and offprint requests to: B. Maes, MD, PhD, Department of Nephrology, University Hospital Gasthuisberg, B-3000 Leuven, Belgium. Email: bart.maes@uz.kuleuven.ac.be

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Introduction

The increased prevalence of tuberculosis (TB) in the western world during the last two decades necessitates an increased awareness of the need for good screening methods for *Mycobacterium tuberculosis* infection.

Haemodialysis patients are at risk to develop TB disease six to 16 times more frequently than other members of the community due to impaired cellular immunity in chronic renal failure [1–3]. The frequent hospital contacts, the older age and the use of immunosuppressive drugs are other factors explaining the higher prevalence of TB in these patients [4]. Therefore, it is important to evaluate the tuberculin skin test (TST) in this haemodialysis population and to compare it with other screening methods, to optimize the detection of infected persons and to treat the latent TB infection [5]. In patients with a positive TST, there also should be an increased awareness of active TB in the case of aspecific symptoms [6]. Unlike in other European countries, in Belgium BCG vaccination has never been used in the prevention of active TB.

Few data are available on tuberculin skin testing in haemodialysis patients [1,7,8]. Routine TST has not been performed in haemodialysis patients because of high rates of anergy in patients with chronic renal failure. Uraemia alters the macrophageal function, which can lead to anergy for skin tests [9]. The prevalence of anergy among haemodialysis patients nowadays (increased dialysis quality and dose) is unclear and reaction to tuberculin is not necessarily comparable with other anergy skin tests such as mumps or tetanus [1]. Moreover, anergy testing is not recommended for patients who may have a compromised ability to react to TSTs such as HIV-infected persons [10].

In this study, several unresolved issues concerning TST reactions in haemodialysis patients were investigated, such as the influence of the primary renal disease, the use of past/current immunosuppressive therapy or vitamin D. Immunosuppressive agents are suspected to enhance the anergy rate, whereas calcitriol is supposed to restore partly the lymphocyte function in haemodialysis patients with uraemic immune defects [11]. Another unresolved question was the impact of the time on dialysis and of dialysis efficiency on skin anergy. Finding a positive link between a well nourished, well dialysed patient and TST response would provide a possible solution to anergy problems. A possible correlation between the response to the skin test and the response to hepatitis B vaccination was also investigated. The presence of such a correlation would give new insights in anergy, affecting different immunological pathways used by hepatitis B vaccination and the skin test. Since there is a presumed higher anergy rate with low zinc levels, a correlation of serum levels of zinc and the response to TST was explored [12].

The primary aim of this prospective multi-centre study, however, was to evaluate the value of the TST as a screening method for detection of latent *M.tuberculosis* infection in haemodialysis patients.

Subjects and methods

Subjects

Patients were recruited from two haemodialysis centres in Belgium (University Hospital Gasthuisberg KU Leuven, Leuven and Virga Jesse Hospital, Hasselt). At the time of this study, a total of 270 patients were treated in the haemodialysis centres of Leuven and Hasselt. Informed consent was obtained before inclusion in the study. The study protocol was approved by the ethical committee for clinical trials in Leuven and Hasselt. In total, 224 patients were included in the study: 105 in Leuven (47%) and 119 in Hasselt (53%). The remaining patients were excluded because of hospital admission or refusal to participate. One patient dropped out after inclusion because she refused the third and fourth skin test.

The mean age of the subjects was 68.3 ± 13.2 years in both centres (median 72 years; range 21–92 years).

The proportion of male patients was 58% (130 patients). Overall, 95% of the patients were born in Belgium, The Netherlands or Germany (countries with low TB prevalence) [13] and 5% were born in other countries with a higher risk for TB (Congo, Indonesia, Romania, Hungary, Morocco and Turkey).

Methods

All patients were investigated for previous TB history, TB contact, awareness of positive TST and BCG vaccination. Unlike other European countries such as the UK, Belgium never used generalized BCG vaccination for the prevention of TB. Patient files were searched for medical history: renal diagnosis, time on dialysis, and for current/past medication (vitamin D supplements and immunosuppressive drugs like corticosteroids, cyclophosphamide, calcineurin inhibitors, azathioprine and mycophenolate mofetil). Recent laboratory findings were taken from the medical records. Dialysis efficiency was measured using Kt/V and PRU. Serum albumin and PCR were used as markers of the patient's nutritional status. Serum levels of zinc (normal values 80–120 μg/dl) were noted. Hepatitis B surface antibody levels were combined with the dose of hepatitis B vaccination administered during the last 3 years (obtained from the medical records) to identify the patient's response to vaccination and to compare it with the response to TST. A chest X-ray, taken within the last year, was examined for TB lesions by an independent pulmonologist and scored blindly as positive, intermediate or negative [14]. Dense pulmonary nodules with or without visible calcification in the hilar area or upper lobes or pleural scarring were scored as positive findings. Lesions that could not undoubtedly be attributed to old TB, however suspected, were considered as intermediate.

Skin testing was performed in September and October 2001. Testing was not done in patients admitted in the hospital to rule out influence of intercurrent active infections.

A TST with 2 tuberculin units of polysorbate 80 (Tween-80) stabilized purified protein derivate (PPD) RT23 (Statens Seruminstitut, Copenhagen, Denmark) was administered on the volar side of the forearm contralateral to the vascular access, using the intracutaneous method (Mantoux) [15]. Testing with 2 tuberculin units PPD RT23 WHO (used most frequently outside the USA) is equivalent to testing with 5 tuberculin units Seibert (used in the USA) [16]. One physician in each centre evaluated the induration by palpation and measured this induration with a flexible ruler 72 h later [17]. All TSTs were placed by skilled nurses.

A positive TST result was defined as an induration of $\geq$10 mm, and in the booster TST an increase of $\geq$6 mm, causing a mimimal induration of 10 mm [18]. All TST-negative patients received a booster TST 7 days later, ~10 cm away from the previous intracutaneous injection. The test was performed and interpreted in the same way. A second and third booster was given with a 7 days interval to the patients not responding to the previous tests [18].

Statistical analysis

The incidence of the positive TST results in the different age groups was calculated using univariate analysis (SAS: PROC UNIVARIATE). Analysis of risk factors for positive TST was performed using correlation analysis (SAS: PROC CORR: Kendall Tau b), univariate analysis (SAS: PROC NPAR1WAY: Mann–Whitney–Wilcoxon/Kruskall–Wallis and SAS: PROC FREQ: Mantel–Haenszel chi^2) and multivariate analysis [SAS: PROC LOGIST: stepwise logistic regression analysis (backward elimination procedure and forward selection procedure)] [19].

For risk factor analysis the following parameters were considered: age, sex, origin (country of birth with low/high TB risk), previous TB history, previous TB contact, previous BCG vaccination, previous positive TST; dialysis-related risk factors: dialysis centre (Leuven, Hasselt), time on dialysis and dialysis efficiency (Kt/V and PRU); nutritional status measured by serum albumin levels and PCR; primary renal disease, classified into eight categories [glomerulonephritis, nefroangiosclerosis, diabetic nephropathy, polycystic kidneys, interstitial nephritis (refluxnephropathy or nephritis due to analgesic abuse), infection, renal carcinoma and unknown

origin]; the current/past use of immunosuppressive drugs (corticosteroids, cyclophosphamide, calcineurin inhibitors, azathioprine and mycophenolate mofetil); the use of vitamin D, serum levels of zinc; recent chest radiograph (scored as positive, negative or intermediate for old TB lesions), response to hepatitis B vaccination (HbsAB, cumulative dose of vaccination the last 3 years). For multivariate analysis, only factors that were significantly different ($P < 0.05$) in univariate comparison were retained.

Results

Demographic data and primary renal disease are summarized in Table 1. None of the patients included was found to have active TB. In Figure 1, the cumulative percentage of positive reactions to four sequential TST is shown. After the first test, 14.7% of the patients showed a positive reaction; the second test added 13.1% more TST-positive patients, whereas the third and the fourth TST only resulted in an additional 4.2 and 4.4% new positive patients, respectively, reaching a total of 32.6% of the patients with a positive TST.

The mean induration of the positive TST was 16 ± 4 mm in test 1, 15 ± 3 mm in test 2, 13 ± 3 mm in test 3 and 13 ± 2 mm in test 4, which gives an overall mean induration of 14.3 mm. There was no significant difference in induration between the four tests and the positive TST after boosting always resulted in an induration of ≥ 10 mm.

Table 2 shows that positive TST results were significantly but weakly correlated with the anamnestic data of a previous TB disease and a previously positive TST. For test 1, origin of the patients was weakly correlated with TST positivity ($P = 0.05$; $R = 0.13$), but no significant correlation could be found between origin and the result of the cumulative four tests. As shown in Tables 3 and 4, there was no significant correlation with age, sex, centre of dialysis, dialysis efficiency or time on dialysis, nutritional state, primary renal disease, immunosuppressive medication, the use of vitamin D, serum levels of zinc and response to hepatitis B vaccination. There was no significant correlation between positive TST and chest radiograph results (old TB lesions were present in 57.8%, 25.7% were without TB lesions, 16.5% had possible TB lesions). In addition to the 73 patients with a positive TST result, there were 34 patients with negative TST but a clearly positive chest X-ray. Using both screening methods, 107 patients (47.8%) were identified as having been infected with *M.tuberculosis*.

Univariate comparison of patients with a negative TST (when cumulating the results of the four tests) *vs* patients with a positive TST, showed a significantly higher number of patients with previous TB disease ($P = 0.0002$) and previously positive TST ($P = 0.0123$) in the positive test group (Table 4). A history of positive

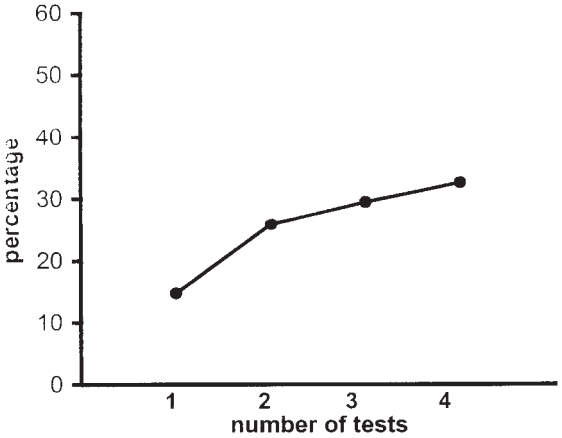

Fig. 1. Cumulative percentage of positive TST after repeated tests.

Table 1. Demographics and primary renal disease

		N/224	%
Centre	Leuven	105	46.9
	Hasselt	119	53.1
Sex	Men	130	58.0
	Women	94	42.0
Origin	Belgium + Netherlands + Germany	213	95.1
	Other (high risk TBC)	11	4.9
Renal diagnosis	Glomerulonephritis	48	21.4
	Vascular renal disease	43	19.2
	Diabetic nephropathy	44	19.6
	Polycystic kidneys	17	7.6
	Interstitial nephritis	33	14.7
	Infection	10	4.5
	Malignancy	1	0.5
	Unknown	28	12.5

Table 2. Correlation analysis between positive TST results (cumulative result of the four tests) and risk factors

	Kendall Tau b	*P*-value
Centre	–0.01	0.82
Sex	–0.09	0.18
Age	0.03	0.62
Origin	0.05	0.48
Previous TB disease	0.25	0.0002
Previous TB contact	–0.04	0.53
Previous TB vaccination	–0.06	0.40
Previous positive TST	0.17	0.01
Renal diagnosis	–0.12	0.07
Immunosuppressive medication		
Corticosteroids	0.04	0.58
Cyclophosphamide	–0.05	0.48
Calcineurin inhibitors	0.06	0.36
AZA/MMF	–0.03	0.61
Other	–0.06	0.40
Time on dialysis	0.03	0.64
Kt/V	0.04	0.53
PRU	–0.05	0.48
Albumin	0.04	0.60
PCR	–0.06	0.38
Vitamin D	–0.03	0.66
Zinc	–0.06	0.42
HbsAB	0.01	0.85
Hepatitis B vaccination	0.02	0.81
Chest radiograph	–0.01	0.92

Table 3. Descriptive statistics in the different subgroups (median and interquartile range): negative TST *vs* positive TST when cumulating the results of the four tests

	Negative	Positive	*P*-value
Total number	151	73	
Age (years)	71 (61–77)	72 (61–77)	0.90
Time on dialysis (years)	2.57 (1.14–4.88)	2.6 (0.95–5.56)	
Kt/V	1.49 (1.33–1.67)	1.51 (1.35–1.71)	0.30
PRU	71 (65–75)	70.8 (65.25–75.15)	0.97
Albumin (g/l)	35.2 (33–37.35)	35 (33.2–37)	0.98
PCR	1 (0.87–1.18)	0.96 (0.82–1.1)	0.19
HbsAB	98 (15–327.75)	55 (7.75–270.25)	0.59
HB vaccination	80 (0–160)	80 (0–200)	0.58
Zinc	77 (69–86)	75 (68.75–83)	0.27

Table 4. Descriptive statistics in the different subgroups (*n* patients): negative TST *vs* positive TST when cumulating the results of the four tests

	Negative	Positive	*P*-value
Number	151	73	
Centre (Leuven/Hasselt)	70/81	35/38	0.82
Sex (male/female)	83/68	47/26	0.18
Origin (TB risk) (low/high)	145/6	68/5	0.48
Renal diagnosis			0.07
Glomerulonephritis	31	17	
Vascular	24	19	
Diabetes	29	15	
Polycystic kidneys	14	3	
Interstitial	22	11	
Infection	8	2	
Malignancy	1	0	
Unknown	22	6	
Previous TB disease (no/yes)	146/5	60/13	0.0002
Previous TB contact (no/yes/possible)	127/22/2	63/10/0	0.53
BCG vaccination (no/yes)	146/5	72/1	0.40
Previous positive TST (no/yes)	151/0	70/3	0.01
Corticosteroids (no/previous/current)	124/14/12	57/10/6	0.57
Cyclophosphamide (no/previous/current)	141/8/1	70/3/0	0.48
Calcineurin inhibitors (no/previous/current)	141/9/0	67/5/1	0.36
AZA, MMF (no/previous/current)	131/11/0	69/4/0	0.60
Other immunosuppressants (no/previous/current)	145/5/0	72/1/0	0.40
Vitamin D (no/yes)	57/93	30/43	0.66
Rx thorax (negative/positive/intermediate)	88/34/27	38/22/9	0.92

BCG vaccination did not correlate with positive TST, but the number of previously vaccinated patients was low, since generalized BCG vaccination was never applied in Belgium.

In a multivariate analysis the only significant risk factor was the presence of previous TB disease (odds-ratio = 6.0 and $P = 0.004$), both in forward selection and in backward elimination procedures.

Discussion

In this study, the usefulness of TST to detect previous *M.tuberculosis* infection was tested in a western European haemodialysis population. Due to their frequent hospital contacts and their old age, haemodialysis patients might have a higher rate of previous *M.tuberculosis* infection [4]. Furthermore, they are at increased risk to develop active TB disease due to the uraemic immunological defect [1–3].

We found a 15% rate of TST positivity, which is comparable with other studies performed on haemodialysis patients in USA and Canada, as far as the results of the first test are concerned [1,7]. However, the cumulative rate after four repeated tests increased to 32.6%.

There are two possible reasons for this relatively high rate of positive TST after repeated testing. The first reason is the booster phenomenon, which has been observed already in a non-dialysis elderly population, when TST was performed four times consecutively [10,14]. A second reason for this higher positive TST results compared with previous studies in the USA and Canada [1] is the fact that in Europe TST positivity in the elderly population is higher than in a comparable Northern American population [1,14].

When evaluating risk factors that can influence TST results, a significant but weak correlation was noted between a positive TST and the anamnestic data of previous TB, and previously positive TST. However, no correlation was found with dialysis efficiency and nutritional status of the patient [20]. Other risk factors such as primary renal disease, immunosuppressive drugs and the use of vitamin D also did not significantly influence the test results. However, the negative result for immunosuppressive drugs (other than corticosteroids) can be related to the rather small numbers in the patient group.

No correlation was found between the reaction to the TST and other immunological pathways, known to be affected by the uraemic immunological defect, as shown by the comparison with the efficacy of hepatitis B vaccination. Low serum levels of zinc were suggested to induce higher anergy rates, but this could not be observed in this study.

No correlation was found between chest radiography and TST result. This could be explained by the high anergy rate in haemodialysis patients, causing a false negative TST with a positive chest X-ray. On the other hand, chest radiography could be false negative due to the presence of only small scars, or false positive due to sequel lesions of previous pulmonary problems other than TB in this elderly population. Since there was no correlation between chest radiography and TST, none of them can be used as the 'gold standard', neither can other tests be excluded for screening *M.tuberculosis* infection in haemodialysis patients. When applying both screening methods, up to 47.8% of the haemodialysis patients were found to be infected with *M.tuberculosis*. This figure underlines the importance of a high index of suspicion for TB infection in this patient population, and hence for the possible development of active TB disease.

The test results were compared with a historical study group of non-ESRD elderly patients [14]. In 223 subjects older than 65 years without active TB, the number of positive reactors increased progressively with the number of performed tests with an overall doubling of the positive TST from test 1 (29%) to test 4 (57%). An age-dependent declining rate of additional reactors with the number of tests was observed. In haemodialysis patients with a mean age of 68.3 years, less positive reactions on the first test were found with a clear levelling off after the second test. The results were not age dependent (see Table 1); there was also no difference in the reaction to TST of the haemodialysis patients when divided in two age groups (15–65 and +65 years), and although the two populations were not identical, the non-ESRD elderly may serve as a good external reference. Unlike in non-ESRD subjects, there is a clear levelling off after the second TST, so that in the haemodialysis population two consecutive tests seem to be sufficient to rule out the booster effect when looking for *M.tuberculosis* infections.

It can be concluded that tuberculin skin testing is a useful tool in detecting latent TB infection in haemodialysis patients, regardless of the immunosuppressive medication, dialysis efficiency or nutritional state, but taking into account that the anergy rate remains higher than in a non-ESRD patient population. We recommend that two consecutive TSTs combined with a chest X-ray should be performed in every haemodialysis patient at the start of dialysis to detect those patients with latent *M.tuberculosis* infection. Tuberculin skin testing and chest radiography should be repeated every year or whenever active TB disease is diagnosed in the unit in order to detect active TB, often without the typical symptoms, at an earlier stage in this high risk population. This could reduce morbidity and mortality due to *M.tuberculosis* infection in chronic haemodialysis patients.

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Conflict of interest statement. None declared.

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