A NEW PREDICTIVE FACTOR FOR THE OUTCOME OF RENAL TRANSPLANTATION

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Summary

The cell mediated immunity (CMI) of a group of patients on regular haemodialysis was measured using a modified dinitrochlorobenzene (DNCB) skin test. The strength of the reaction was graded from 0 to 15 on an objective scale which we called the DNCB index. This index was much reduced in the dialysis patients in comparison with a group of healthy controls. Thirty-six dialysis patients were subsequently transplanted and graft survival was assessed at six months. A significantly higher graft failure rate was observed in those with a strong skin reaction than in those with a weak or absent response (P < 0.01). While the mean DNCB is much lower than normal in dialysis patients, there is a wide variation within this group. We have found that the DNCB index correlates well with renal allograft survival suggesting that this skin test has value in the prediction of transplant outcome.

Introduction

It is commonly suggested that the main factors which determine the outcome of human kidney transplantation are the quality of the donor kidney, the adequacy of immunosuppression and the use of tissue typing. Yet one of the earliest observations in patients with chronic renal failure on regular haemodialysis was that they would accept skin allografts for extended periods [1], showing that they had profound depression of cell-mediated immunity. Later studies have also shown that they have poor skin reactions to memory antigens such as PPD and streptokinase/streptodornase (SK/SD) [2]. DNCB is an antigen to which very few people have been exposed and forms the basis of probably the best single skin test for measurement of CMI. We have used a quantitative modification of this test in a group of patients on regular haemodialysis and related the results to the outcome of subsequent transplantation.
Patients and Methods

Thirty-six patients satisfied the criteria that the skin reaction to DNCB had been measured while on regular haemodialysis and follow-up after cadaver transplantation exceeded six months. Patients who received live donor transplants, whose grafts failed for technical reasons or who died within six months with functioning grafts had been excluded.

There were 24 males and 12 females with a mean age of 32 years and age range of 17 to 48 years. The causes of renal failure were glomerulonephritis (27), chronic pyelonephritis (6), hypertensive nephrosclerosis (1), horseshoe kidney (1) and polycystic kidneys (1). The mean duration of haemodialysis prior to transplantation was 28 months with a range of 5 to 102 months. At the time of transplantation 29 of the patients were attending hospital for dialysis while the remaining 7 were receiving treatment at home. Dialysis was carried out three times per week with 8 hour sessions for those at home and 5 hours for those attending hospital. All received a high calorie diet containing between 50 and 80 grams of protein depending on body weight. None were on drugs known to affect the immune response.

HLA typing was performed prospectively in all cases and the number of antigens mismatched was as follows: no mismatch – 7 patients; one mismatch – 13 patients; 2 mismatches – 12 patients; 3 mismatches – 3 patients; 4 mismatches – one patient.

Immunosuppression consisted of prednisolone and azathioprine. The skin reaction to DNCB was related to graft survival at 6 months. It was considered that this gave a fairly accurate reflection of the success rate as rejection is much less common beyond this time.

Figure 1. Diagram of DNCB method. The numbers indicate the dose in µg of DNCB applied to the forearm for the initial sensitisation (left) and testing 14 days later (right)
The method used for the DNBC test is illustrated in Figure 1. Sensitisation was carried out with 2000 µg dissolved in 0.1 ml acetone applied to an area of the forearm 2.5 cm in diameter. Fourteen days later, the patient was tested with 5 doses of DNBC containing 30, 15, 7.5, 3.7 and 1.8 µg respectively each applied on a one cm diameter felt pad*. The reaction was scored at 48 hours thus:

0 : no reaction or erythema only.
1 : erythema and induration confined to the patch.
2 : erythema and induration extending beyond the patch.
3 : as for 2 with blistering in addition.

The DNBC index was calculated as the sum of the scores for each patch. Thus it could range from 0 to 15.

Results

The DNBC index for the 36 patients is shown in Figure 2 with the results for 15 healthy controls. The mean index for the patients was 2.5 compared with 9.1

![Graph showing DNBC index in control subjects and dialysis patients](image)

Figure 2. DNBC index in a group of control subjects and dialysis patients. The large open circles indicate the mean for each group.

* All test strips supplied by Amercolab, Sweden
for the controls, the difference being significant (P < 0.001). The 7 home dialysis patients had a higher mean index (3.1) than those attending hospital (2.3) but this difference was not significant. Twenty of the 36 grafts were functioning at 6 months. It can be seen from Figure 2 that the DNCB index tended to be higher in those patients whose grafts failed. The mean index in this group was 4.2 compared to 1.1 for the surviving group, a significant difference (P < 0.02).

In the lefthand column of Table I the six month graft survival is compared in the 27 patients with weak or negative DNCB reaction (index 0–3) and the 9 with a stronger reaction (index > 3). The weak reactors had a graft survival of 70% compared to 11% for those with a strong reaction, the difference being significant (P < 0.01).

**TABLE I. Relationship of DNCB Index and HLA Matching to 6 month Graft Survival**

<table>
<thead>
<tr>
<th>DNCB index</th>
<th>Irrespective of HLA</th>
<th>Graft survival at 6 months</th>
<th>2 or more HLA mismatches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt; 2 HLA mismatches</td>
<td></td>
</tr>
<tr>
<td>0 – 3</td>
<td>19 of 27 (70%)</td>
<td>10 of 13 (77%)</td>
<td>9 of 14 (64%)</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>1 of 9 (11%)</td>
<td>1 of 7 (14%)</td>
<td>0 of 2 (0%)</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The middle and righthand columns of the table show the combined influence of HLA matching and the DNCB index. Well-matched kidneys in patients with DNCB indices of 0–3 showed a slightly but not significantly higher survival than those which were poorly matched. So far, no patient with a poor match and index > 3 has had a functioning graft at 6 months.

**Discussion**

There is ample evidence that uraemic patients have depressed CMI [2–4]. Prolongation of skin allograft survival has been shown to occur in uraemic patients [1] and there is little doubt that uraemic depression of immunity assists renal allograft survival.

The fact that numerous methods of testing CMI have been described suggests that none is entirely satisfactory. The various in vitro tests have the inherent disadvantage of testing the lymphocytes outside their normal environment. Skin transplantation is a complicated procedure and may sensitise to HLA antigens. Most skin tests have used antigens to which the patient may previously have been exposed thus testing not only present immunological status but also immunological memory. We feel that the DNCB skin test avoids all these disadvantages. It is simple and safe and uses a readily available compound. Also it is a new antigen which does not test immunological memory.
and it does not sensitize to HLA antigens. Finally, we have shown it to be reproducible. Of 23 patients who had two or more tests, only one showed a change which would have resulted in a move from one group to the other in the table.

To make the test quantitative we have used 5 different dilutions of DNCB and have thus been able to demonstrate a normal range for a group of healthy controls. As a group, the dialysis patients had a depressed skin reaction to DNCB but there was wide variation with a zero reaction in 47% and results well within the range seen in the controls in 19%. It would be logical to suggest that patients with CMI within normal or approaching normal limits would have much greater likelihood of rejecting their grafts. Rolley et al [5] used the skin reaction to DNCB to classify dialysis patients into reactors and non-reactors and showed a good correlation with the outcome of transplantation. In the present study by using a quantitative approach to the DNCB test we have clearly shown that patients whose grafts subsequently rejected had a greater DNCB response (mean index 4.2) in comparison with those whose grafts survived at 6 months (mean index 1.1). Only one from the group of 9 patients with an index of more than 3 has so far reached 6 months with a functioning graft. This survival rate (11%) compares with 70% for those with an index of 3 or less.

We have confirmed in a recent analysis from our department a relationship between HLA matching and transplant outcome [6]. In this study we therefore combined the HLA and DNCB data to see if this would further increase our ability to predict the outcome, but with the small number of patients in the study so far we were unable to do so.

In conclusion we have shown that quantitative measurement of the skin reaction to DNCB gives an accurate prediction of transplant outcome. With our technique, a DNCB index of more than 3 was associated with a 6 month graft survival of only 11%. Once the results from larger numbers of patients have confirmed these figures, we would suggest that patients with an index of more than 3 should not be transplanted unless in exceptional circumstances. This would not only avoid subjecting these patients to a procedure with only a small chance of success but would also release more cadaver kidneys for the patients most likely to benefit.

References

4. Diamantopoulos, AA, Hamilton, DNH and Briggs, JD. Unpublished observations

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Open Discussion

WOODS (London) Did you find any change in the cell-mediated immunity index with time on dialysis?

DIAMANDOPOULOS We have tested different patients 2, 3 or 4 times and still found roughly the same results. Only one patient changed from the group of low responders to the group of high responders.

COLOMBI (Lucerne) Have you any explanation for the difference between the DNCB index in home patients and in Centre dialysis patients?

DIAMANDOPOULOS I have no explanation for that. The only difference we could find between these two groups of patients was that the home dialysis patients were dialysed 8 hourly thrice weekly as opposed to 5 hours thrice weekly for centre dialysis, and perhaps there is some dialysable factor which suppresses cellular immunity.

FOX (Sheffield) You get a very wide difference between results in patients who have a DNCB index of below 3 and those above 3. There is also a very wide difference in results in patients who have had previous blood transfusions. Have you examined your results for those patients who have and have not had blood transfusions in the past, because this may make quite a large difference?

DIAMANDOPOULOS We have found some negative correlation between the strength of the cell-mediated immunity index and previous transfusion. This was true for frozen cells but not for whole blood. Patients who had a lot of transfusions also had a low DNCB index. It looks as though some factor which depresses cell-mediated immunity also causes severe anaemia.

HANICKI (Cracow) It would be interesting to compare the results of the DNCB reaction in dialysed and non-dialysed patients with the cellular pattern of an open skin window technique, the Roebuck-Crowley test. Did you try such correlation?

DIAMANDOPOULOS No, we did not.