Rapid and Simple Microtest for the Diagnosis of Chronic Granulomatous Disease
(NBT-Test with Candida Albicans)

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Abstract. A slide test is described for the cytochemical determination of NBT-reduction in leucocytes stimulated by phagocytosis of Candida albicans spores. Reliable results were obtained in 198 healthy controls, 12 carriers of and 18 patients with chronic granulomatous disease, with one questionable result in each group.

Key words: Nitroblue tetrazolium (NBT), chronic granulomatous disease (CGD), phagocytosis, neutrophil function.

The diagnosis of chronic granulomatous disease (CGD) can be established reliably by demonstrating a deficiency of activated polymorphonuclear (PMN) leucocytes to reduce Nitroblue-tetrazolium (NBT). We have described a modification of the original method [1-3] in which the barely visible latex particles used for stimulation of phagocytosis were replaced by the big spores of Candida albicans [4]. In the present brief note a micromodification is described needing only one drop of blood and little more than 30 min of time.

Material

Nitroblue-tetrazolium (NBT): The commercially available substance was dissolved in Sörensen's buffer to a concentration of 100 mg/100 ml without any further preparation (M/15, pH = 7.0).

Candida Albicans Preparation

The colonies on the surface of a Petri dish cultured for 24-48 h were suspended in 10 ml of saline, boiled for 10 min, washed twice with saline and resuspended in approximately 1 ml NaCl 0.9%.

Methyl Green

A 2% solution is made in distilled water and washed four times with chloroform.

Method

One drop of whole blood, one drop of NBT solution and one small drop of Candida albicans suspension are carefully mixed on a fat-free microscope slide and incubated in a moist chamber Petri dish (30 min. at 37°C or 90 min. at room temperature). The slide is then put in an upright position on a filter paper, thus allowing all material not fixed to the glass surface to drip down. Alternatively after incubation coagula may be removed with a needle or cotton swab by rotating movements, or small coagula may be gently washed away with one or two drops of saline. The preparations are air-dried, fixed in methanol (1-2 min.) and counterstained with Methyl green (5 min.). Microscopic evaluation is based on the same criteria (phagocytic index and NBT-index) as in the previously described macromethod [4].

Subjects

18 previously diagnosed patients suffering from CGD, 12 heterozygous carriers and 198 healthy controls were tested. The paediatricians caring for the patients took the blood, prepared the slides and sent them to our laboratory under a code number (15 patients, 10 carriers, 13 controls). The code was only broken after evaluation by at least two individuals in our laboratory, and the results compared with those of the macromethod.

Results

Table 1 shows the results of the 228 tests. The previously established criteria [4] seemed useful and valid, with no overlapping between the 3 groups of genetically different individuals.

Table 1. Results of 228 NBT microtests

<table>
<thead>
<tr>
<th>NBT index</th>
<th>N</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18</td>
<td>patients CGD</td>
</tr>
<tr>
<td>45-91%</td>
<td>12</td>
<td>carriers</td>
</tr>
<tr>
<td>98.2% (range: 95-100)</td>
<td>135</td>
<td>controls</td>
</tr>
</tbody>
</table>

Of the 15 coded cases with CGD there was some doubt in one. This, however, was a very special patient who also presented varying numbers of very finely blue-stippled Candida particles in the macrotest. The slides of this patient were judged as doubtful (carrier or patient?). In all the other patients the diagnosis was unequivocal with 0% NBT positive cells.

Amongst the coded 10 carriers, there were 8 correct diagnoses, one was doubtful (carrier or normal?) and
one was mis-diagnosed as a healthy control. On the other hand, of the 13 coded controls one was mistaken as a probable heterozygous carrier.

Summary and Conclusions

A simple and rapid micromodification of the NBT test with phagocytosing PMN leukocytes proved to be reliable in the diagnosis of 17 known cases of CGD, leaving some doubt in one additional, apparently singular, case. It was also useful for the differentiation of heterozygous carriers and healthy controls; in each of these groups there was however one questionable case (1/10 and 1/13). The test can be recommended for screening a great number of patients with septic infections. If the diagnosis of CGD is to be firmly established, further more accurate tests (macromethod of the NBT test and bacterial killing test) are necessary.

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