The Impact of Immunological Parameters on the Development of Phantom Pain after Major Amputation

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Objectives. To investigate the relationship between local and systemic inflammatory markers and phantom limb pain.

Methods. In 39 consecutive patients undergoing major amputations nerve biopsies, serum and clinical data was collected. Patients were followed up for 12 months to report on the incidence and severity of phantom limb pain.

Results. After 12 months, 78% of the surviving patients had phantom pain, the symptom usually commencing within 14 days of operation. The severity of macrophage infiltration within the nerve biopsy was negatively correlated to the inception of phantom pain (P = 0.026). While serum TNF-alpha concentration was positively correlated to mortality (P = 0.021).

Conclusions. The immune status existing before the amputation and the local immunological milieu influence the onset of phantom pain.

Keywords: Phantom pain; Immunological parameters; Prognostic factors; Human nerve tissue.

Phantom pains are neuropathic pain that may occur after amputation of limbs and which are referred to the missing limb or the missing organ.1–5 An extremely wide range of clinical parameters have been claimed to be associated with phantom pain. However, despite numerous studies encompassing various clinical parameters, none of these factors has actually been shown to have any correlation with the occurrence of phantom pain, either in randomised or in independent studies.1 The exact pathogenesis of phantom pain is still unclear, this being one of the main reasons why successful therapy has not yet been discovered.6,7 Experiments in numerous animal models have shown that the immunological component plays an important role in the occurrence of pain.8–10 The purpose of this study was to examine the impact of immunological parameters on the prognosis and pathogenesis of phantom pain.

Materials and Methods

Patients

This study comprised 39 consecutive patients who were treated by a major amputation of the lower limb performed in the Department of Vascular Surgery of the University of Erlangen-Nürnberg during the period from July 2000 to July 2001. Clinical data were recorded prospectively for all these patients, including a pain questionnaire adapted from Kooijman et al.11 Clinical data were recorded preoperatively, on the day of discharge, and thereafter every 3 months until the end of follow-up. In addition, nerve tissue was taken from each patient during the operation, snapped-frozen in liquid nitrogen and stored at −80 °C for subsequent processing.

Immunocytochemistry and quantification

We used the following primary antibodies for immunocytochemistry (anti-human CD3, clone-NCLCD3p), anti-human CD4 (clone-NCL-CD4-1F6), anti-human CD8 (clone-C8/1440), CD20 (clone-L26), CD68 (clone-PG-M1), TNF-alpha (clone-28401.111), TNF-RI (R&D Systems) and HOI (clone-N19) was applied at appropriate dilution (100–200 µl/slide).
Slides were viewed with a 40× objective and an area of the nerve was chosen that was free of artefacts. All immunoreactive cells were counted within a defined field measuring 240×180 μm². Three such fields were analysed.

**ELISA**

Serum was collected from 32 patients on admission to hospital. ELISAs were performed for TNF-alpha, sICAM-1, interleukin-6 (R&D System) and C-reactive protein (C-reactive Protein, Olympus System, Olympus Diagnostica).

<table>
<thead>
<tr>
<th>Phantom pain postoperative</th>
<th>Yes (SD)</th>
<th>No (SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>13 (15.7)</td>
<td>15 (19.4)</td>
<td>0.744</td>
</tr>
<tr>
<td>CD20</td>
<td>2 (6.5)</td>
<td>2 (3.1)</td>
<td>0.893</td>
</tr>
<tr>
<td>CD68</td>
<td>2 (4)</td>
<td>5 (3.3)</td>
<td>0.026</td>
</tr>
<tr>
<td>TNF-α</td>
<td>3 (8.4)</td>
<td>3 (5.5)</td>
<td>0.973</td>
</tr>
<tr>
<td>TNF-α-RI</td>
<td>16 (45.9)</td>
<td>13 (23.2)</td>
<td>0.973</td>
</tr>
<tr>
<td>HOI</td>
<td>8 (13.5)</td>
<td>16 (33.1)</td>
<td>0.328</td>
</tr>
</tbody>
</table>

Each line shows the median number of positive staining cells for each marker, counted in three microscope fields at 20× magnification; TNF-α-RI, tumor necrosis factor-alpha-receptor I; HOI, hemeoxygenase1; SD, standard deviation.

**Statistics**

The clinical data together with the experimental data derived from the immunohistochemical studies and the serum assays were analyzed using SPSS (SPSS for Windows 11.0) databank. The significance level was taken as P<0.05.

**Results**

**Patients and frequency of phantom pain**

A total of 39 patients was enrolled in the study. Twenty-one patients (54%) developed phantom pain within the first 14 days after the operation. From discharge up to the end of follow-up six further patients developed phantom pain. Patients who had no phantom pain at the end of 6 months did not develop any phantom pain up to the end of follow-up at 12 months.

**Correlation between routine preoperative laboratory parameters and the occurrence of phantom pain**

The routine preoperative laboratory investigations comprised full blood count, clotting parameters, LDL, HDL, Quick, PTT and creatinine, were not correlated with the occurrence of phantom pain.

**Correlation between immunological parameters and phantom pain**

Soluble ICAM, C-reactive protein (CrP), interleukin-6, TNF-alpha, TNF-α-RI and HOI were of no value for predicting phantom pain (data not shown). Immune cells were demonstrable in almost every nerve biopsy removed at the time of amputation. T-cells (CD3+) were most frequently identified, with smaller number of B-cells (CD20) and macrophages (CD68) also seen. Macrophages were less frequently identified in the
nerves removed from patients who subsequently developed phantom pain \((P=0.026)\) (Table 1) (Fig. 1).

**Prognostic markers for survival after a major amputation**

In the first 3 months after amputation 20% of the patients died. At the end of follow-up after 12 months, only 27 (67%) of the patients who had had a major amputation were still alive. Because of the high mortality of 33% at 12 months, we examined various clinical and immunological parameters to assess their value for predicting survival. Other concomitant diseases were of no relevance for 1-year survival. Surviving patients had serum levels of TNF-alpha which were about 50% lower \((P=0.021, \text{Mann–Whitney} \text{ } U \text{ } \text{test})\). sICAM, interleukin-6 and CrP showed no association with survival. The occurrence of phantom pain did not correlate with survival (Table 2).

**Discussion**

Patients who have to undergo a major amputation have increased morbidity and mortality after the operation. The principal morbidity is phantom pain, which has a prevalence of up to 78%.\(^{1,2}\) In our series, phantom pain usually began within the first 14 days. Data from animal experiments suggest that immunological regulatory circuits are of critical importance for the inception of chronic pain.\(^{8–10}\) As phantom pain is a chronic pain phenomenon beginning immediately after the amputation,\(^{1}\) it seems reasonable to suppose that immune reactions within nerves, initiated even before the amputation, might influence the disease process.

In nerves from phantom pain patients, fewer macrophages (CD68+ cells) were demonstrable than in nerves from patients who did not develop any phantom pain. Numerous studies in experimental animals have shown that macrophages play a central role in the induction of chronic pain.\(^{9,10}\) In our investigations of human tissues the appearance of macrophages had a protective effect against the onset of phantom pain. Conceivably, by the production of antioxidants such as glutathione, macrophages may help to protect nerves against free radicals through the production of antioxidants such as glutathione.\(^{9,10}\)

In this study, we also identified an association between serum TNF-alpha concentrations at the time of operation and subsequent survival. This association likely reflects this cytokine as a marker of atherosclerosis burden or associated co-morbidities.\(^{12,13}\)

Our data suggests an influence of local inflammation on the development of phantom pain. Further studies are required to clarify the mechanisms that may be involved.

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**References**


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