Research Paper

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Protective role of natural killer cells in neuropathic pain conditions

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Abstract

During the past few years, the research of chronic neuropathic pain has focused on neuroinflammation within the central nervous system and its impact on pain chronicity. As part of the ERA-Net NEURON consortium, we aimed to identify immune cell patterns in the cerebrospinal fluid (CSF) of patients with herpes zoster neuralgia and patients with polyneuropathy (PNP), which may contribute to pain chronicity in these neuropathic pain conditions. Cerebrospinal fluid of 41 patients (10 herpes zoster and 31 PNP) was analyzed by flow cytometry identifying lymphocyte subsets: $CD4^+$ (T-helper cells), $CD8^+$ (cytotoxic T cells), $CD19^+$ (B cells), and $CD56^+$ (natural killer [NK]) cells. At baseline and at follow-up, the somatosensory phenotype was assessed with quantitative sensory testing. In addition, the patients answered epidemiological questionnaires and the PainDETECT questionnaire. Immune cell profiles and somatosensory profiles, as well as painDETECT questionnaire scores, were analyzed and correlated to determine specific immune cell patterns, which contribute to chronic pain. We found a negative correlation ($P = 0.004$, $r = -0.596$) between the frequency of NK cells and mechanical pain sensitivity (MPS), one of the most relevant quantitative sensory testing markers for central sensitization; a high frequency of NK cells correlated with low MPS. The analysis of the individual follow-up showed a worsening of the pain condition if NK-cell frequency was low. Low NK-cell frequency is associated with signs of central sensitization (MPS), whereas high NK-cell frequency might prevent central sensitization. Therefore, NK cells seem to play a protective role within the neuroinflammatory cascade and may be used as a marker for pain chronicity.

Keywords: Cerebrospinal fluid, Chronicity, Sensitization, Zoster, Polyneuropathy

1. Introduction

Chronic pain is a widespread phenomenon in modern Western society—and a serious problem for healthcare systems. About one-fifth of the European population is affected by chronic pain and is impaired at home or at work.[6] However, only 2% of the patients who require treatment by specialized pain therapists are treated adequately.[6,40] Results of a representative sample in Germany show that 31.9% of patients with impairing pain were treated by a pain specialist underlining the current lack of therapists.[21]

Seven to 18% of the population[5] remain in pain after the initial disease has healed and reach a stage of chronic neuropathic pain.[1] Apart from certain risk factors for chronic pain (such as lower socioeconomic status,[39] depression,[31,48] and history of abuse or job dissatisfaction[37,39]), it is well known that the immune system has a major impact on the development of pain after nerve injury. The most important mechanisms of cellular immune response are inflammation and cytotoxicity.[13,32,33,49]

Nerve injuries often lead to chronic neuropathic pain associated with peripheral and central neuroimmunological activation and inflammatory responses of nerve tissue.[1] Inflammatory mediators promote the activation of immunocompetent nerve cells (eg, B and T cells), sensitize afferent neurons, and lead to hyperalgesia,[29] which is symptomatic for neuropathic pain. In this process, a maladaptive immune response may promote permanent pain.[7] Furthermore, a suppression of the immune response within the context of nerve injury can prevent the development of hyperalgesia.[10]

Austin and Moalem-Taylor demonstrated that after nerve lesions, both the innate and the adaptive immune systems are decisively involved: Peripheral nerve injuries provoke reactions in the immune system, such as the infiltration of inflammatory cells (eg, T cells) or the activation of resident immune cells (eg, mast cells and microglia).[1]

Previous animal studies demonstrated an association between specific immune cell patterns within the cerebrospinal fluid (CSF) and (persistent) pain conditions, finding leukocyte trafficking into the spinal cord after peripheral L5 nerve transsection, which correlated with mechanical allodynia.[28,35,45]

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.painjournalonline.com).

PAIN 162 (2021) 2366–2375

http://dx.doi.org/10.1097/j.pain.0000000000002274

Immunological markers of pain chronicity are of high value to identify patients with a higher risk for pain chronicity at an earlier stage and allow modifying treatment strategies. In humans, the immune cell pattern within the CSF has not been investigated with respect to chronic neuropathic pain conditions so far.

Two of the pain conditions predisposing the development of chronic pain are herpes zoster neuralgia and polyneuropathy (PNP). Specific treatment remains challenging, and the intake of pain medication is often required for years.

As part of the ERA-Net Neuron consortium, we analyzed the immune cell profile within the CSF in correlation with the somatosensory phenotype in these specific neuropathic pain disorders. Because the CSF mainly contains T, B, and natural killer (NK) cells,[43,44] we concentrated on these cells in the flow cytometry (FACS) analysis. The aim of the project was to identify immune cell markers for chronic pain.

2. Methods

2.1. Study design

Ten patients suffering from herpes zoster neuralgia and 31 patients with PNP were examined from June 2016 up to January 2019.

During baseline examination, patients received the painDETECT questionnaire (PDQ) as well as quantitative sensory testing (QST) in the most affected area and the corresponding contralateral side (patients with zoster) or on both sides of the most affected area (patients with PNP) to assess the somatosensory profile.

Cerebrospinal fluid was analyzed according to clinical routine diagnostics including cell counts, protein, lactate, and glucose. In addition, a FACS analysis of the CSF was performed. Lymphocyte subsets were analyzed using the following antibodies (with fluorochromes) from BD Biosciences: anti-CD45 (PerCP-Cy 5.5), anti-CD56 (PE), anti-CD3 (FITC), anti-CD19 (APC), anti-CD4 (PE-Cy7), and anti-CD8 (APC-Cy7). All analyses were performed using a BD FACSCanto analyzer (BD Biosciences), and data were analyzed using FlowJo (version 10.6.2). Only samples with at least 500 lymphocytes (defined by $CD45^+$ expression) were included into the analysis. Quality control for doublet exclusion and live/dead cell staining was performed exemplary, which showed neither doublets nor a relevant amount of apoptotic cells (no CSF sample exceeded 100 cells/μL). Most importantly, CSF samples were processed within 30 minutes after a lumbar puncture. For these samples, the frequencies of $CD3^+CD4^+$ cells (T-helper cells), $CD3^+CD8^+$ cells (cytotoxic T cells), $CD19^+$ cells (B cells), and $CD56^+$ cells (NK cells) were assessed (**Fig. 1**) and further correlated with somatosensory profiles. Three months later, QST and PDQ were repeated to identify pain chronicity in patients with zoster and patients with PNP. All patients gave their written informed consent to participate in the study. The study is registered at German Clinical Trials Register (Registration trial: DRKS00023537) and was performed in accordance with the Declaration of Helsinki and approved by the local ethics committee (ID: D552/15).

2.2. Interview and questionnaire

Demographic data such as age, sex, duration of disease, and affected area were assessed.

2.3. Pain intensity and characteristics

Presence of pain, pain location, and severity (numeric rating scale ranging from 0 [no pain] to 10 [maximum intensity]) were assessed by means of an interview.

2.4. Pain characteristics

PainDETECT[15] is a screening tool to identify a neuropathic pain component in patients with chronic pain. It includes questions about general pain intensity, pain development, and possible radiating pain. It also asks for the presence and intensity of typical neuropathic symptoms. Finally, an end score is calculated to quantify whether a neuropathic pain component is unlikely, uncertain, or likely. The PDQ score is already used as a progression parameter and has proven to be valid and reliable.

2.5. Quantitativ sensory testing

Quantitative sensory testing was used to assess the patient's somatosensory profile. It is a standardized measurement that tests the somatosensory function of primary afferent nerve fibers (Aβ, Aδ, and C fibers) and their central pathways.

Quantitative sensory testing was performed in accordance to the protocol of the German Research Network on Neuropathic Pain (Deutscher Forschungsverbund Neuropathischer Schmerz, DFNS).[17,34,52] Testing was performed in the affected dermatome and the corresponding contralateral side in patients with zoster and on both sides in the most affected area in patients with PNP (ie, dorsum of the feet [n = 29] and dorsum of the hand [n = 2]).

The following 13 parameters were recorded: cold detection threshold (CDT), warm detection threshold (WDT), thermal sensory limen (TSL), cold pain threshold (CPT), heat pain threshold (HPT), pressure pain threshold (PPT), mechanical pain threshold (MPT), mechanical pain sensitivity (MPS), wind-up ratio (WUR), mechanical detection threshold (MDT), vibration detection threshold (VDT), dynamic mechanical allodynia (DMA), and paradoxical heat sensation (PHS).

2.6. Statistical analysis

The analysis of the collected data was performed using IBM SPSS statistics for Mac (version 26.0).

The QST results were analyzed according to the current guidelines and compared with a reference database of healthy controls.[27,51]

Individual QST parameters of the 2 groups were compared using the Mann–Whitney *U* test. The intraindividual comparisons from the first and second examinations were analyzed using the Wilcoxon test.

Aiming to find associations between immune cell distribution within the CSF and the sensory phenotype, we performed the Spearman correlation analysis. In a first step, we used an exploratory approach without correction for multiple testing. In a second step, we identified robust results using Bonferroni correction, including FACS data, PDQ scores, and QST markers for pain chronicity (ie, HPT, as marker for peripheral sensitization,[25] and MPS, MPT, and WUR, as markers for central sensitization[3]); P values <0.05 were considered as statistically significant.

3. Results

The epidemiological data of patients with herpes zoster and patients with PNP are shown in **Table 1**.

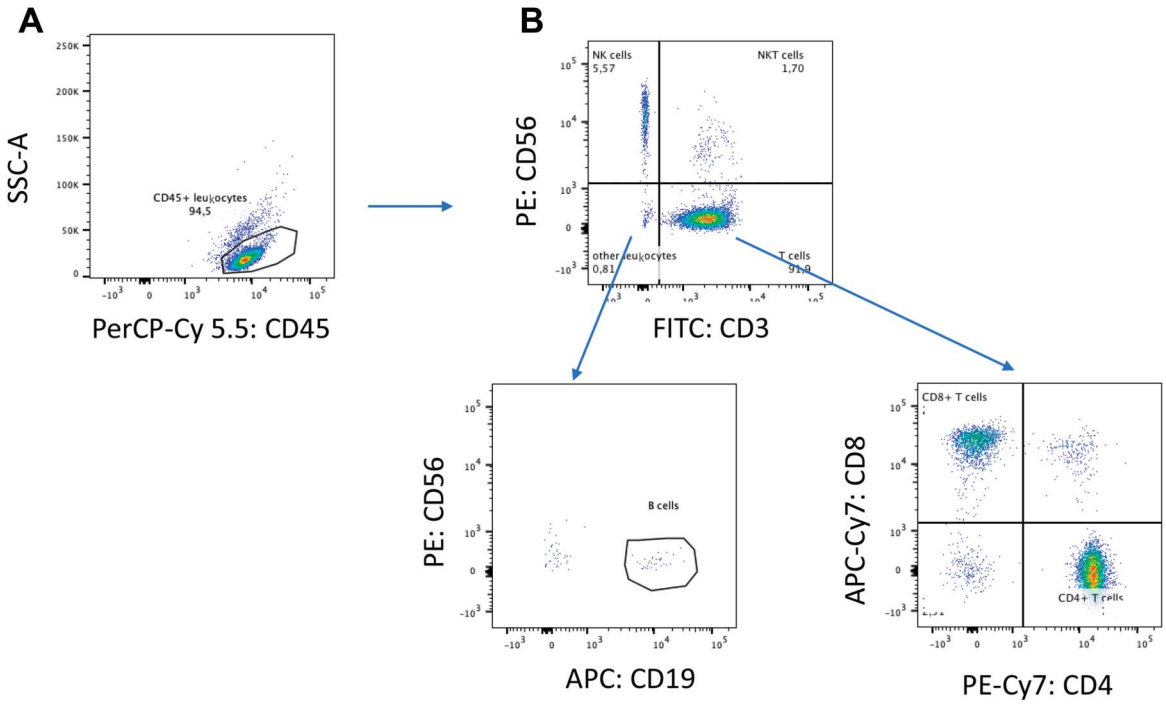

Figure 1. Gating strategy for flow cytometric analysis in the cerebrospinal fluid. Leukocytes were identified by CD45 expression (A) and next, the NK-cell and T-cell population was identified (B). T cells were further subdivided into $CD4^+$ T helper and $CD8^+$ cytotoxic T cells (D), whereas B cells were identified according to CD19 expression from CD3-negative and CD56-negative lymphocytes. NK, natural killer.

3.1. Comparison of pain intensity and painDETECT questionnaire scores of the 2 patient groups at baseline and follow-up

The pain ratings and PDQ scores are shown in **Table 2**. The 2 patient groups did not differ significantly in pain parameters (minimum and maximum pain; pain intensity within the previous 72 hours) or PDQ scores ($P > 0.1$ for all comparisons). There were no differences between baseline and follow-up assessment of pain and PDQ scores ($P > 0.5$ for all comparisons).

3.2. Comparison of somatosensory profiles of the 2 patient groups at baseline and follow-up

Regarding the QST parameters, differences were found between the patients with zoster and the patients with PNP regarding WDT (WDT Z-values zoster: −0.23 ±1.72 vs WDT Z-values PNP: −1.63 ±1.34, $P = 0.03$), TSL (TSL Z-values zoster: −0.7 ±1.51 vs TSL Z-values PNP: −1.75 ±1.15, $P = 0.025$), CPT (CPT Z-values zoster: 0.6 ±1.04 vs CPT Z -values PNP: −0.36 ± 1.02, $P = 0.012$), HPT (HPT Z-values zoster: 0.83 ±1.98 vs HPT Z-values PNP: −0.89 ±1.17, $P = 0.004$), and VDT (VDT Z-values zoster: −1.07 ±2.33 vs VDT Z-values PNP: −3.43 ±2.54, $P = 0.021$) (**Fig. 2**), indicating a pronounced function loss of fibers mediating temperature, touch, and vibration in patients with PNP.

Comparing follow-up data with baseline, no significant changes in QST findings were found, neither in patients with zoster ($P > 0.05$ for all comparisons) nor in patients with PNP ($P > 0.1$ for all comparisons) (**Fig. 3**).

3.3. Clinical routine cerebrospinal fluid analysis of patients with herpes zoster and patients with polyneuropathy

The CSF findings of all patients are shown in **Table 3**. Apart from the leukocyte count (entire cohort: 18.11/μL ± 26.6/μL at zoster vs 1.9/μL ± 2.4/μL at PNP; $P = 0.000436$; FACS cohort: 23.33/μL ± 32.15/μL at zoster vs 2.5/μL ± 3.2/μL at PNP; $P = 0.04$; **Fig. 4**), the CSF findings did not differ significantly between the 2 groups of patients with zoster and patients with PNP ($P > 0.05$ for all comparisons).

3.4. Flow cytometry analysis

Following strict inclusion criteria, each CSF sample had to contain at least 500 lymphocytes to undergo further analysis, resulted in the inclusion of 22 eligible FACS data.

Table 1

Epidemiological data of patients with herpes zoster and patients with polyneuropathy.

	Patients with herpes zoster	Patients with PNP
Number [n](%)	10 (24)	31 (76)
Age	63.2 y (±14.34)	65.19 y (±14.5)
Gender		
Female [n](%)	4 (40)	12 (39)
Male [n](%)	6 (60)	19 (61)
Duration of disease [± SD]	1.7 mo (±1.14)	48.98 mo (±79.62)
Affected area		
Face [n](%)	2 (20)	0 (0)
Arm [n](%)	2 (20)	0 (0)
Hand [n](%)	1 (10)	2* (6)
Trunk [n](%)	4 (40)	0 (0)
Foot [n](%)	0 (0)	29 (94)
No affected area [n](%)	1 (10)	0 (0)

* Both patients suffer from a chronic inflammatory demyelinating polyneuropathy (CIDP), in both cases the hands are much more affected than the feet.
PNP, polyneuropathy.

Table 2

Pain ratings and PDQ scores of patients with herpes zoster and patients with polyneuropathy.

	Patients with herpes zoster		Patients with PNP	
	Baseline	Follow-up	Baseline	Follow-up
Pain intensity within the previous 72 h [mean ± SD] (range)	4.44 ± 3.36 [0-9], N 9	2.33 ± 2.07 [0-5], N 6	3.16 ±3.53 [0-10], N 31	2.9 ± 2.7 [0-8], N 30
Minimal pain [Mean ± SD] (range)	3.8 ± 3.9 [0-9], N 5	2.0 ± 2.45 [0-5], N 6	1.0 ±1.79 [0-7], N 28	1.31 ± 2.04 [0-7], N 26
Maximum pain [Mean ± SD] (range)	6.4 ± 4.34 [0-10], N 5	3.67 ± 3.39 [0-10], N 6	4.21 ±3.99 [0-10], N 28	4.67 ± 3.79 [0-10], N 30
PDQ total score [Mean ± SD] (range)	14.89 ± 6.75 [4-22], N 9	9.2 ± 4.09 [2-12], N 5	10.52 ± 8.03 [0-26], N 31	10.57 ± 7.5 [0-25], N28
PDQ evaluation: neuropathic pain component unlikely/uncertain/likely [n](%)	3 (33)/2 (22)/4 (44)	5 (100)/0 (0)/0 (0)	19 (61)/6 (19.5)/6 (19.5)	17 (61)/6 (21)/5 (18)

All values are depicted as mean ± SD [minimum–maximum], number of patients.
PNP, polyneuropathy; PDQ, painDETECT questionnaire.

Epidemiological parameters as well as pain intensity within the previous 72 hours, minimal pain and maximum pain, of patients with eligible FACS data were comparable with the entire study cohort and are shown in **Tables 4 and 5**.

The exact distribution of the different cells in the CSF is shown in **Table 6**. There were no significant differences between patients with zoster and patients with PNP regarding cell distribution within the CSF ($P > 0.5$ for all comparisons).

The PDQ score indicated a significant difference between patients with zoster and patients with PNP (PDQ score zoster: 17.2 ± 6.02 vs PDQ score PNP: 8.63 ± 7.75; $P = 0.04$); ie, patients with zoster reported symptoms that made a neuropathic pain component more likely more often than patients with PNP.

Apart from that, the 2 groups did not differ in pain characteristics ($P > 0.1$ for all comparisons). In addition, there were no significant differences between baseline and follow-up assessment of pain and PDQ scores ($P > 0.5$ for all comparisons).

3.4.1. Correlation analysis of immune cell frequencies and painDETECT questionnaire scores

There were no significant correlations between the FACS data and PDQ scores for all patients or in any of the subgroups ($P > 0.05$ for all comparisons).

3.4.2. Correlation analysis of immune cell frequencies and somatosensory parameters

3.4.2.1. All patients

Cytotoxic T cells ($CD8^+$) correlated positively ($r = 0.482$, $P = 0.023$, n = 22) with HPT, ie, increased $CD8^+$-cell frequency was associated with heat hyperalgesia.

There was a negative correlation ($r = -0.596$, $P = 0.004$, n = 21) between MPS and the frequency of NK cells: Increased NK-cell frequency correlated with a reduced MPS. This finding remained robust after Bonferroni correction (adjusted $P = 0.036$) (**Fig. 5**).

3.4.2.2. Patients with herpes zoster

At baseline, a positive correlation was found between $CD8^+$-cell frequencies and MPS ($r = 0.990$, $P = 0.001$, n = 5). This finding remained robust after Bonferroni correction (adjusted $P = 0.009$) (**Fig. 5**).

3.4.2.3. Patients with polyneuropathy

A high $CD8^+$-cell frequency was associated with a reduced MPS ($r = -0.516$, $P = 0.041$, n = 16) as well as increased heat hyperalgesia ($r = 0.551$, $P = 0.027$, n = 16).

There was a significant correlation ($r = -0.545$, $P = 0.029$, n = 16) between MPS and the frequency of NK cells. An increased NK-cell frequency correlated with a reduced MPS.

3.5. Correlation analysis of immune cell frequencies and pain outcomes

We found no significant correlations between FACS data and PDQ scores in the follow-up ($P > 0.05$), and there was neither any significant change in PDQ scores between baseline and follow-up ($P > 0.1$).

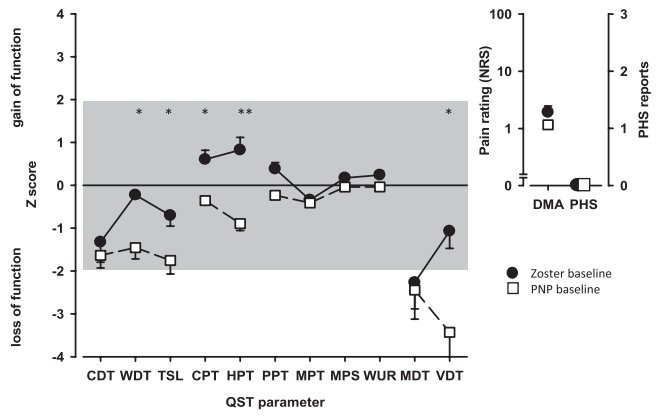

Figure 2. Comparison of baseline QST findings of patients with herpes zoster and patients with PNP. Z -values of the 13 QST parameters are given here. The gray area indicates the range of normative values according to the database of the German Research Network on Neuropathic Pain (DFNS). Error bars indicate the standard error of the mean. Z-value = each individual parameter is related to its region-specific, age-specific, and sex-specific reference range and is displayed as the number of SDs above or below the normal mean value. CDT, cold detection threshold; CPT, cold pain threshold; DMA, dynamic mechanical allodynia; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PHS, paradoxical heat sensation; PNP, polyneuropathy; PPT, pressure pain threshold; QST, quantitative sensory testing; TSL, temperature sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

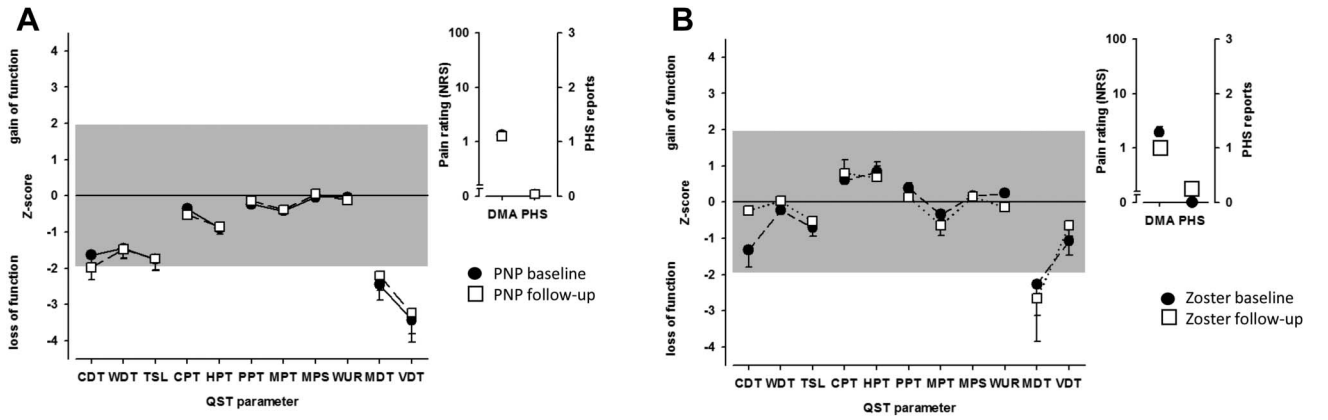

Figure 3. Comparison of baseline and follow-up QST findings of (A) patients with PNP and (B) patients with herpes zoster. Z-values of the 13 QST parameters are given here. The gray area indicates the range of normative values according to the database of the German Research Network on Neuropathic Pain (DFNS). Error bars indicate the standard error of the mean. Z-value = each individual parameter is related to its region-specific, age-specific, and sex-specific reference range and is displayed as the number of SDs above or below the normal mean value. CDT, cold detection threshold; CPT, cold pain threshold; DMA, dynamic-mechanical allodynia; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PHS, paradoxical heat sensation; PNP, polyneuropathy; PPT, pressure pain threshold; TSL, temperature sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

In 2 patients, the PDQ revealed that a neuropathic pain component was uncertain at baseline but had become likely at the follow-up examination. Both patients showed low NK-cell frequencies compared with the mean of all eligible NK frequency data.

Two patients developed an abnormal HPT at follow-up assessment; no valid CSF results are available for either patient.

There were 4 patients whose initial normal MPS values became abnormal over time. Two patients had valid FACS findings and showed a reduced NK-cell frequency compared with the mean.

One patient developed an abnormal WUR over time and showed reduced NK-cell frequency compared with the mean.

4. Discussion

This study aimed to find specific immune cell patterns within the CSF, which can contribute to pain chronicity.

We found a significant association of NK-cell frequency and MPS, an important QST marker for central sensitization and therefore a potential marker for chronic pain. These results indicate a protective effect of NK cells regarding pain chronicity. We also found significant correlations regarding cytotoxic T cells, which at first glance seemed contradictory. In patients with zoster, $CD8^+$-cell frequency correlated with MPS (high levels of $CD8^+$ cells were associated with pronounced central sensitization). However, in patients with PNP, $CD8^+$-cell frequency was associated with a reduced MPS, indicating less signs of central sensitization. These findings are discussed as follows:

4.1. Protective effect of natural killer cells on central sensitization

The most interesting result was a significant inverse correlation between MPS (as one of the most relevant markers for central

Table 3

Cerebrospinal fluid findings of all patients.

	Unit	Reference range	Herpes Zoster	Polyneuropathy
Leukocyte count	/μL	<5	5.46 ± 13.69 [1-76], N 10	3.61 ± 8.19 [0-51], N 31
Glucose	mmol/L	2.8-4.4	3.47 ±0.32 [3.04-3.89], N 10	3.79 ±0.56 [2.96-5.72], N 31
Glucose quote L/S		>0.5	0.66 ± 0.06 [0.52-0.76], N 9	3.85 ± 17.84 [0.4-100], N 31
Protein (total)	mg/L	150-450	485 ± 237.98 [255-963], N 10	500.87 ± 182.74 [229-936], N 31
Albumin	mg/L	35-53	297.1 ± 207.76 [39.1-679], N 10	308 ± 118.19 [105-628], N 31
Lactate	mmol/L	1.3-2.4	1.8 ±0.26 [1.46-2.25], N 10	1.78 ± 0.3 [1.34-2.71], N 31
IgA liquor	mg/L	0.7- 4.0	4.6 ±2.68 [0.84-9.82], N 10	5.32 ±7.14 [0.84-39.7], N 31
IgG liquor	mg/L	7-16	31.14 ± 14.82 [16.7-57.9], N 10	49.74 ± 63.17 [10.1-294], N 31
IgM liquor	mg/L	0.4 – 2.3	0.87 ± 1.73 [0-5.73], N 10	0.94 ± 0.45 [0.25-2.25], N 31
Q albumin	$*10^{-3}$		7.68 ± 4.28 [3.22-15.9], N 10	7.76 ± 3.37 [2.95-16.7], N 31
Q IgG	$*10^{-3}$		3.8 ± 1.81 [1.76-7.2], N 10	4.74 ± 5 [1.26-23], N 31
Q IgA	$*10^{-3}$		2.27 ± 1.27 [0.9-4.36], N 10	2.04 ± 1.2 [0.7-6.29], N 31
Q IgM	$*10^{-3}$		0.68 ± 0.5 [0.16-1.41], N 10	0.55 ± 0.62 [0.09-2.81], N 30

All values are depicted as mean ± SD [minimum–maximum], number of patients.
CSF, cerebrospinal fluid.

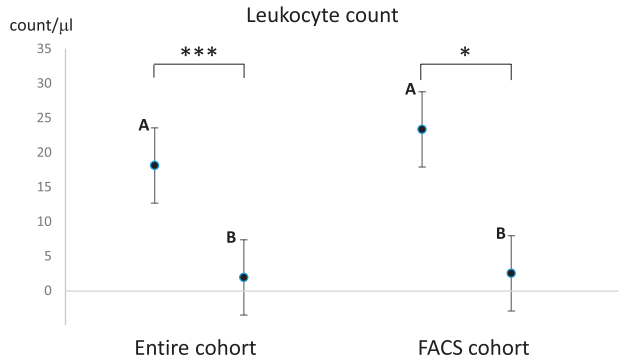

Figure 4. Comparison of the leukocyte counts of patients with zoster and patients with PNP. The CSF results of the entire cohort as well as the FACS cohort are shown. (A) Patients with herpes zoster (B) patients with PNP. Entire cohort: $P = 0.000436$; FACS cohort: $P = 0.04$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. CSF, cerebrospinal fluid. PNP, polyneuropathy; QST, quantitative sensory testing.

sensitization in the QST[22]) and the frequency of NK cells. This indicates that NK cells might have a protective effect on pain sensitization.

Natural killer cells belong to the innate immune system and their primary function is destroying tumor-infected or virus-infected cells. However, it has become more and more clear that their function comprises much more. There are few studies that demonstrate their recruitment in the periphery after nerve lesions. Rats with injured nerves showed a significant upregulation of NK cells in these nerves compared with sham-operated controls.[11] In another experiment in mice, it could be shown that the injury of peripheral nerves led to the recruitment of NK cells into these nerves. The function of NK cells correlated with a reduced incidence of hypersensitivity. The authors consider this selective NK-cell-mediated destruction of damaged axons as a supplement to Wallerian degeneration and point to the therapeutic potential of NK cells in painful neuropathy by clearing partially damaged nerves.[12] Moreover, Gao et al. showed that NK cells were increased both in their activity and their quantity in the spleen and peripheral blood after electrical stimulation of ligatured sciatic nerves in rats. Because of repetitive electroacupuncture, IL-2 and β-EP, 2 efficacious activators of NK cells, were increased.[16]

The infiltration of immune cells into the central nervous system during peripheral neuroinflammation is discussed controversially.[28] After peripheral nerve transection, increased spinal activity of $CD4^+$ and MHC-II cells accompanied by persistent mechanical allodynia was observed,[46] which points to a consecutive central neuroinflammatory response. Another study showed the trafficking of $CD3^+$ T lymphocytes and MHC-II cells into the spinal cord correlating with mechanical allodynia.[45] Rutkowski et al. demonstrated extravasation of microglia in the central nervous system of rats suffering from neuropathic pain due to L5 peripheral spinal nerve transection. The authors suggest a specific role of infiltrating cells with neuroprotective or antihyperalgesic effects.[35] Our demonstrated reduction of MPS in combination with an increased NK-cell frequency suites these findings and provides the first data in humans. By contrast, recent studies could not find any evidence for an infiltration of peripheral immune cells into the spinal cord parenchyma after peripheral injury.[19,47] These studies transected peripheral nerve tissue within an animal model, without finding an immune cell migration into the central nervous system. The latter studies investigated the spinal cord parenchyma, whereas in our study the CSF was analyzed. Notably, immune cell infiltration into the CSF does not necessarily equal immune cell infiltration into the spinal cord. The next scientific step should be the comparison of immune cell activity within the CSF and spinal cord parenchyma during neuroinflammation to enlighten this topic.

Table 4

Epidemiological data of patients with eligible FACS data.

	Patients with herpes zoster	Patients with PNP
Number [n](%)	6 (27)	16 (73)
Age	58.5 y (±12.74)	58.63 y (±14.46)
Sex		
Female [n](%)	2 (33)	6 (38)
Male [n](%)	4 (67)	10 (62)
Duration of disease [± SD]	1.63 mo (±1.28)	45.02 mo (±70.06)
Affected area		
Arm and shoulder [n](%)	2 (33)	0 (0)
Trunk [n](%)	4 (67)	0 (0)
Foot [n](%)	0 (0)	16 (100)

PNP, polyneuropathy.

Known QST markers that indicate (peripheral or central) sensitization are HPT, MPS, WUR, and MPT. There were 4 patients who showed sensitization as their initial normal MPS values became abnormal over time. Two of them had valid FACS findings; both showed reduced NK-cell frequencies in the FACS analysis. In addition, there was one patient who developed an abnormal WUR. This patient also showed a reduced NK-cell frequency compared with the mean of NK-cell frequencies of the entire study cohort. These findings also support the assumption that NK cells could have a protective effect on pain sensitization. These observations should of course be reevaluated in a larger cohort of subjects.

There is ample evidence that NK cells are involved in the pathogenesis of herpes zoster.[4,14,30,53] NK cells circulate in the blood and migrate into inflammated tissue as part of the innate immune response and thus react early to infection.[50] NK cells are reported to contain herpes zoster infection. On the contrary, Campbell et al. showed that the varicella-zoster virus specifically penetrates healthy NK cells and thereby contributes to the spread of the infection.[8]

4.2. Cytotoxic T cells and sensitization

In patients with zoster, high $CD8^+$-cell levels were associated with *pronounced* central sensitization (MPS), whereas in patients with PNP, $CD8^+$-cell frequency was associated with *less signs* of central sensitization.

These findings suggest that in the acute stage of a neuropathic pain condition (within our study cohort herpes zoster neuralgia occurred within the first 3 months) cytotoxic T cells promote central sensitization, most probably as a relevant motor within the neuroinflammatory cascade.

Recent studies were able to identify $CD8^+$ cells as one of the major cell groups infiltrating ganglia after or during zoster infection.[18,41,42] Today, it is known that VZV antigens induce T-cell-mediated immune responses.[24,36] In a murine spinal model, Cao et al. reported that T-lymphocyte-deficient mice presented a reduction in injury-induced hypersensitivity, suggesting T lymphocytes as an important factor for the maintenance of neuropathic pain.[9]

On the contrary, in a chronic neuropathic pain condition (our cohort of patients with chronic painful PNP exceeded

Table 5

Questionnaire results (baseline and follow-up).

	Patients with herpes zoster		Patients with PNP	
	Baseline	Follow-up	Baseline	Follow-up
Pain intensity in the previous 72 hours	4.83 ± 3.97 [0-9], N 6	3 ± 2.65 [0-5], N 3	2.56 ± 3.41 [0-10], N 16	2.38 ± 2.55 [0-8], N 16
Minimal pain	3.80 ± 3.90 [0-9], N 5	2.33 ± 2.52 [0-5], N 3	0.86 ± 1.56 [0-5], N 14	0.94 ± 1.69 [0-4], N 16
Maximum pain	6.40 ± 4.34 [0-10], N 5	4.67 ± 5.03 [0-10], N 3	4.2 ± 2.09 [0-10], N 15	4.25 ± 3.82 [0-10], N 16
PDQ total score	17.2 ± 6.02 [7-22] N 9	6.5 ± 6.36 [2-11] N 5	8.63 ± 7.75 [0-24], N 16	9.79 ± 8.66 [0-25], N 14
PDQ evaluation: neuropathic pain component unlikely/uncertain/likely [n](%)	3 (33)/2 (22)/4 (44)	5 (100)/0 (0)/0 (0)	19 (62)/6 (19)/6 (19)	17 (61)/6 (21)/5 (18)

All values are depicted as mean ± SD [minimum–maximum], number of patients.
PNP, polyneuropathy; PDQ, painDETECT questionnaire.

pain onset by years), $CD8^+$-cell levels were associated with *reduced* central sensitization. These findings suggest that $CD8^+$ cells develop protective features when neuropathic pain becomes chronic. What underlines this assumption is the result of a murine study concerning *chronic* arthritis: Their results indicate that $CD8^+$ cells have a protective and analgesic effect on inflammatory pain by the release of endogenous opioids in the chronic stage of disease.[2] In addition, another murine experiment showed that $CD8^+$ cells are necessary for the recovery of paclitaxel-induced or cisplatin-induced mechanical allodynia and they abate spontaneous pain and numbness.[26] In chronic pain conditions such as complex regional pain syndrome and fibromyalgia, a reduction in circulating $CD8^+$ T cells within the blood has been found.[23]

This switch from promoter to protector is known for some cytokines and immune cells: Within the immune response they can have both a promoting and a protective character. However, we do not know when and why this switch occurs and what causes it. Improved knowledge of this could be of therapeutic importance because it could give an indication of the progress of a disease and might help to adapt therapeutic strategies.

4.3. Leukocyte trafficking into the central nervous system

The leukocyte cell count of the patients with zoster was significantly higher than in patients with PNP. This result indicates possible leukocyte migration into the central nervous system during peripheral inflammation within the spinal nerve root. An increase of the leukocyte cell count within the spinal cord due to varicella-zoster infection has been previously reported.[38] The authors explain these CSF changes by anatomical proximity of the affected ganglia to the central nervous system. Haanpää et al. also suggested direct spread of the varicella virus from the dorsal root ganglion into the central nervous system.[20] Leukocyte trafficking into the central nervous system remains a controversial topic on which little is known so far.

5. Limitations

As a pilot study on human CSF-FACS-analysis in patients with zoster and patients with PNP, the study's main limitation is the relatively low number of patients. Because we had to define strict quality criteria for inclusion into the FACS analysis, the number of cases with valid FACS results was markedly smaller than the original group size. Furthermore, drawing CSF from patients is an invasive procedure that needs a clear neurological indication, which is not always the case in acute zoster or PNP. Thus, the extraction of CSF was limited because of ethical reasons and only possible when indicated.

6. Conclusion

Several studies in mice reported a connection between NK cells and pain sensitization. No evidence has yet been found that frequency and activity level of NK cells correlate with loss of sensitivity in human CSF. Our study was the first to show that a high NK-cell frequency in the human CSF is associated with reduced central sensitization (MPS) in neuropathic pain disorders. Thus, NK cells could be a marker for central pain sensitization and therefore a possible marker for chronic pain. Despite the fact that NK cells represent only a small fraction of immune cells, they seem to play a unique role regarding the immune response after nerve injury.

Although our observations were drawn from a limited number of patients, we found promising markers for pain chronicity within the CSF, which withstood correction for multiple testing and which are worthwhile to be considered in future CSF studies in a larger cohort (eg, multicenter trials).

Table 6

Distribution of the different cells in the CSF

	Patients with Zoster at baseline	Patients with PNP at baseline
CD4 in %	58.56 ± 12.74 [36.72-71.82], N 6	51.65 ± 18.92 [15.77-77.79], N 16
CD8 in %	14.4 ± 11.06 [1.07-31.16], N 6	13.3 ± 9.05 [1.64-32.23], N 16
CD19 in %	3.54 ± 5.72 [0.32-15.14], N 6	4.01 ± 7.2 [0.43-30.06], N 16
CD56 in %	7.11 ± 6.48 [2.2-19.4], N 6	6.06 ± 4.56 [0.16-17.93], N 16

All values are depicted as mean ± SD [minimum–maximum], number of patients.
CSF, cerebrospinal fluid; PNP, polyneuropathy.

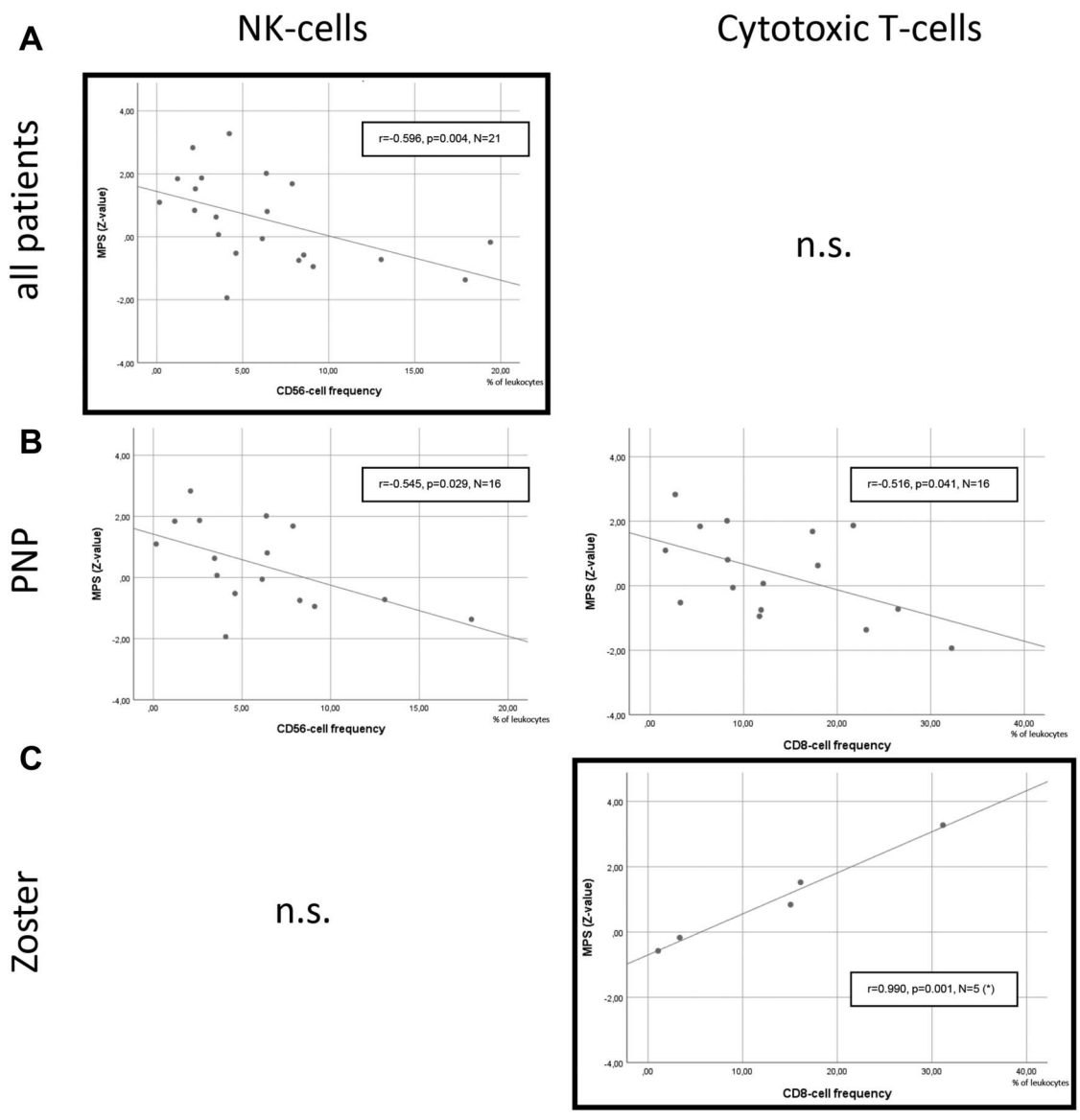

Figure 5. Significant correlations of QST parameters with immune cells. (A) All patients, (B) patients with PNP, and (C) patients with herpes zoster. The figures with black outline remained significant after Bonferroni correction. *One patient with zoster wished to terminate the examination prematurely. HPT, heat pain threshold; MPS, mechanical pain sensitivity; n.s., not significant; PNP, polyneuropathy; QST, quantitative sensory testing.

Conflict of interest statement

J. Lassen received financial support from Pfizer OFG Germany GmbH, outside the submitted work. K.H. Stürner reports personal fees from Biogen GmbH, personal fees from Hoffmann La Roche AG, personal fees from Sanofi Genzyme, personal fees from Bayer, and personal fees from Merk KGaA, outside the submitted work. J. Gierthmühlen reports personal fees from TAD Pharma, Lilly, Novartis, and Grünenthal, outside the submitted work. F. Leypoldt reports grants from Germany Ministry of Research and Education, grants from ERA-NET through German Research Council, personal fees from Grifols, personal fees from Roche, personal fees from Biogen, personal fees from Alexion, and personal fees from Novartis, outside the submitted work. R. Baron reports grants from German Federal Ministry of Education and Research (BMBF): Member of the ERA-NET NEURON/IM-PAIN project (01EW1503), during the conduct of the study; grants from EU projects: "Europain" (115007), DOLORisk (633491), IMI-Paincare (777500), German Federal Ministry of Education and Research (BMBF): Verbundprojekt: Frühdetektion von Schmerzchronifizierung (NoChro) (13GW0338C), German Research Network on Neuropathic Pain (01EM0903), Pfizer Pharma GmbH, Genzyme GmbH, Grünenthal GmbH, Mundipharma Research GmbH und Co KG., Novartis Pharma GmbH, Alnylam Pharmaceuticals Inc, and Zambon GmbH. He reports personal fees from Pfizer Pharma GmbH, Genzyme GmbH, Grünenthal GmbH, Mundipharma, Sanofi Pasteur, Medtronic Inc. Neuromodulation, Eisai Co Ltd, Lilly GmbH, Boehringer Ingelheim Pharma GmbH & Co KG, Astellas Pharma GmbH, Desitin Arzneimittel GmbH, Teva GmbH, Bayer-Schering, MSD GmbH, Seqirus Australia Pty. Ltd, Novartis Pharma GmbH, TAD Pharma GmbH, Grünenthal SA Portugal, Sanofi-Aventis Deutschland GmbH, Agentur Brigitte Süss, Grünenthal Pharma AG Schweiz, Grünenthal B.V. Niederlande, personal fees from Pfizer Pharma GmbH, Genzyme GmbH, Grünenthal GmbH, Mundipharma Research GmbH und Co. KG, Allergan, Sanofi Pasteur, Medtronic, Eisai, Lilly GmbH, Boehringer Ingelheim Pharma GmbH&Co.KG, Astellas Pharma GmbH, Novartis Pharma GmbH, Bristol-Myers Squibb, Biogenidec,

AstraZeneca GmbH, Merck, Abbvie, Daiichi Sankyo, Glenmark Pharmaceuticals S.A., Seqirus Australia Pty. Ltd, Teva Pharmaceuticals Europe Niederlande, Teva GmbH, Genentech, Mundipharma International Ltd. United Kingdom, Astellas Pharma Ltd. United Kingdom, Galapagos NV, Kyowa Kirin GmbH, Vertex Pharmaceuticals Inc., Biotest AG, Celgene GmbH, Desitin Arzneimittel GmbH, Regeneron Pharmaceuticals Inc. USA, Theranexus DSV CEA Frankreich, Abbott Products Operations AG Schweiz, Bayer AG, Grünenthal Pharma AG Schweiz, Mundipharma Research Ltd. United Kingdom, Akcea Therapeutics Germany GmbH, Asahi Kasei Pharma Corporation, AbbVie Deutschland GmbH & Co. KG, Air Liquide Sante International Frankreich, Alnylam Germany GmbH, Lateral Pharma Pty Ltd, Hexal AG, and Angelini, Janssen, outside the submitted work. P. Hüllemann reports grants from German Federal Ministry of Education and Research (BMBF) and grants from Zambon GmbH, outside the submitted work. The remaining authors have no conflicts of interest to declare.

Acknowledgments

This study was supported by the German Federal Ministry of Education and Research (BMBF): Member of the ERA_NET NEURON/IM-PAIN Project (grant number: 01EW1503). We thank Stephen McMahon and Franziska Denk for their valuable scientific input.

Supplemental video content

A video abstract associated with this article can be found at http://links.lww.com/PAIN/B347.

Article history:
Received 16 December 2020
Received in revised form 15 February 2021
Accepted 2 March 2021
Available online 24 March 2021

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