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Determination of Paraneoplastic neuropathy in newly diagnosed breast tumor patients

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ABSTRACT

Objective: The presence of paraneoplastic neuropathy in newly diagnosed breast tumor patients will be investigated. Aim of study is conduce of early diagnosis of the disease and new biomarkers responsible for the pathogenesis to be identify.

Materials and Methods: Thirty-two patients admitted to the Oncology outpatient clinic with newly diagnosed breast cancer were included in the study. After the neurological examination of the patients, Lanss neuropathic pain scale and blood tests were performed. Before chemotherapy all patients underwent electromyography (EMG). Two tubes of 5 cc of venous blood were obtained by screening onconeuronal antibodies.

Results: Patients included in the study; 1 (3.1%) grade 1, 14 (43.8%) grade 2, 17 (53.1%) grade 3 invasive breast cancer was diagnosed. Perineural invasion was detected in 5 (15.6%) patients. Progesterone receptor positivity was found in 26 (81.2%) patients and estrogen receptor positivity was found in 27 (84.4%) patients. In 7 (21.9%) patients, CERBB2 was positive for Ki 67 in 25 (78.1%) patients. Neuropathic findings were present in 6 (18.8%) patients. Sensory neuropathy was detected by electrophsiologic tests in only 2 (6.2%) patients. A total of 12 (37.5%) patients had onconeuroneal antibody positivity. Antibody positivity was significantly higher in patients with high grade tumor ($p = 0.008$).

Conclusion: Paraneoplastic neuropathies can be confused with neuropathies due to non-cancerous causes both clinically and electrophysiologically. When approaching paraneoplastic neuropathies, pathological findings should be carefully reviewed and evaluated with other findigs.

It should be remembered that an underlying breast tumor may be present in women with cancer-related neuropathic complaints.

1. Introduction

Paraneoplastic neurological syndromes (PNS) are rare neurological conditions in patients with cancer, which may affect one or more parts of the nervous system, independent of the local or direct effect of the underlying malignancy. PNS cannot be explained by the underlying cancer-related metastasis, opportunistic infections and side effects of cancer treatment and is believed to be mostly triggered by autoimmune mechanisms [1–4].

PNS can occur between 1/1000 and 1/10000 of cancer patients. These syndromes are often associated with small cell lung cancer (SCLC), ovarian cancer, breast cancer, thymoma and lymphoma [2,5]. PNS usually manifests before the diagnosis of cancer and for this reason the recognition of these syndromes is very important both for the control of symptoms and for the detection and treatment of the underlying cancer [1,6].

Breast cancer is the most common type of cancer and the second most common cause of death in women [7]. The main PNS associated with breast cancer are subacute cerebellar degeneration, retinopathy, opsoclonus-myoclonus syndrome, sensory neuropathy and stiff-man syndrome [8]. On the other hand, onconeuronal antibodies are positive in only 60–70% of breast cancer related PNS. Although the presence of anti-neuronal antibody is helpful in the diagnosis of PNS, its absence does not exclude an autoimmune etiology [9].

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Cancer-related neuropathies may occur due to infiltration of the peripheral nevre with tumor cells, infections, treatment side effects or PNS. All types of peripheral neuropathy (demyelinating, axonal, motor, sensory or autonomic) may occur in association with cancer [10].

The development of new treatment modalities has had a positive effect on the survival of cancer patients. Consequently, prevalence of cancer-related neurological problems has increased. Despite recent advances in cancer physiopathology, mechanisms of cancer-related neuropathy are still unknown. Also, we have insufficient knowledge about the impact of cancer-related factors (e.g. lymph node metastasis, molecular expression profile of the tumor tissue, histologic subtype etc.) on occurrence of paraneoplastic neuropathy in breast cancer. The aim of this study was to identify the prevalence of paraneoplastic neuropathy and associated clinical/oncological features in breast cancer patients and to determine antibody-based biomarkers associated with breast cancer related paraneoplastic neuropathies.

2. Materials and methods

2.1. Patients

Thirty-two consecutive patients admitted to the Oncology outpatient clinic with newly diagnosed breast cancer were included in the study. After the neurological examination of the patients, neuropathic pain was investigated with LANSS neuropathic pain scale and blood tests were performed. None of the patients had used toxic substances or drugs that could explain neurological findings. Patients with coexisting neurological or systemic disorders were also not included.

Before chemotherapy all patients underwent electromyography (EMG) including unilateral median and ulnar nerve motor and sensory conduction studies in the upper limb and bilateral tibial and peroneal motor and sural sensory conduction studies in the lower limbs [11]. Reference values for sensory nerves are given in Table 1 and reference values for motor nerves are given in Table 2.

2.2. Antibody tests

Two tubes of 5 cc of venous blood were obtained by screening onconeuronal antibodies. After 20 min at room temperature, the blood was centrifuged at 2000 rpm for 10 min and sera were stored in aliquots in a − 80 °C freezer until use. Immunoblot sticks containing recombinant proteins of target paraneoplastic antigens of Hu, Yo, Ri, Ma2, CV2, amphiphysin, Tr (DNER), Zic4, Sox1, titin, recoverin and glutamic acid decarboxylase (GAD)65 were used for detection of serum onconeural antibodies.

2.3. Statistical analysis

Statistical analysis were performed by IBM Statistical Package for Social Sciences (SPSS) 21 package program. Categorical data were expressed as frequency (n) and percentage (%) and continuous data were expressed as mean and standard deviation. Chi-square test was used for the analysis of categorical variables, and Fisher's exact test was used in cases where the chi-square test assumptions were not met. p value smaller than 0.05 were evaluated as statistically significant. The power of the research is in post hoc power analysis; $n = 32$, effect size = 0.5 Df = 1, the power of the selected study was calculated as 80%. Gpower was calculated by using 3.1.9.2.

Table 1
Reference values of sensory nerves examined [11].

Nerve	Age 30 Amplitude (μv)	Age 50 Amplitude (μv)	Age 70 Amplitude (μv)	Conduction Speed (m/Sn)
Median	24	14	9	45
Ulnar	18	11	7	45
Sural	6	1	–	40

3. Clinical features and pathological results

The clinical and demographic characteristics of the patients are detailed in Table 3. All patients were women aged 30–65 years. In the pathological staging of patients with invasive breast cancer; 1 (3.1%) was grade 1, 14 (43.8%) were grade 2 and 17 (53.1%) were grade 3. Pathological examination revealed perineural invasion in 5 (15.6%) patients. Progesterone receptor positivity was found in 26 (81.2%) patients and estrogen receptor positivity was found in 27 (84.4%) patients. 7 (21.9%) patients had CERBB2 and 25 (78.1%) patients had Ki 67 positivity. Only 2 (6.2%) patients had sensory neuropathy on EMG. Neurological examination revealed neuropathic findings in 6 (18.8%) patients. LANSS score was over 12 in 4 (12.5%) patients.

4. Onconeuronal antibody results

Onconeuronal antibody positivity was observed in 12 (37.5%) of the patients included in the study. Antibody positivity is detailed in Table 4.

The Relationship Between the Presence of Immunohistochemical Findings and Antibody Positivity in Patients.

Onconeuronal antibody positivity was detected in 11 (40.7%) estrogen receptor positive cases and 16 (59.3%) estrogen receptor positive cases were found to be antibody negative. There was no significant relationship between the presence of estrogen receptor and antibody positivity ($P = 0.62$). Antibody positivity was detected in 11 (42.3%) cases positive for progesterone receptor, while antibody positivity was detected in 15 (57.7%) cases positive for progesterone receptor. No significant correlation was found between the presence of progesterone receptor and antibody positivity ($P = 0.37$).

Antibody positivity was detected in 2 (28.6%) cases positive for C-erbB-2, while antibody positivity was found in 5 (1.4%) cases positive for c-erbB-2. There was no significant relationship between c-erbB-2 positivity and antibody positivity ($p = 0.68$). Antibody positivity was found in 8 (32%) patients who were positive for Ki-67, while antibody negativity was found in 17 (68.0%) patients who were positive for Ki-67. There was no significant relationship between ki-67 positivity and antibody positivity in the subjects included in the study. ($P = 0.37$).

Antibody positivity was detected in 3 (40.0%) cases with perineural invasion, while antibody negativity was detected in 2 (40%) cases with perineural invasion. There was no significant relationship between the presence of perineural invasion and antibody positivity. ($P = 0.35$).

5. Relationship between tumor grade and antibody positivity

In the pathological staging of patients with invasive breast cancer; 1 (3.1%) was grade 1, 14 (43.8%) were grade 2 and 17 (53.1%) were grade 3. The correlation between tumor grade and antibody positivity was evaluated and grade 1 and 2 tumors were evaluated together. Tumor grade was grade 1–2 in 2 (16.6%) patients with antibody positivity and grade 3 in 10 (83.4%) patients with antibody positivity. A statistically significant correlation was found between antibody positivity and tumor grade. Antibody positivity was significantly higher in patients with high grade tumors. ($p = 0.008$) (Table 5).

6. Characteristics of two cases with sensory neuropathy

Sensory neuropathy was detected in EMG in only two cases. The characteristics of these cases are shown in Table 6 in detail.

7. Discussion

PNS are usually associated with underlying cancer and most of the

Table 2
Reference values of motor nerves examined [11].

Nerve	Age 30 Amplitude (μv)	Age 50 Amplitude (μv)	Age 70 Amplitude (μv)	Conduction Speed (m/sn)	DML (sn)
Median	7,3	5,2	3,7	50	4,6
Ulnar	8,1	7,2	6,4	51	3,5
Peroneal	2,4	0,8	0,2	40	6,0
Tibial	7,3	3,2	1,4	37	5,8

DML: Distal motor latency.

Table 3
Demographic and clinical characteristics of the cases included in the study.

Age (mean ± standard deviation)	46,5 ± 9,08
Gender(n(%))	
Female	32 (%100)
Male	0
Invasive Ductal Breast Cancer (n(%))	
Grade 1	1 (%3,1)
Grade 2	14 (%43,8)
Grade 3	17 (%53,1)
Perineurol invasion (n(%))	
+	5 (%15,6)
−	27 (%84,4)
Progesterone receptor (n(%))	
(+)	26 (%81,2)
(−)	6 (%18,8)
Estrogen receptor (n(%))	
(+)	27 (%84,4)
(−)	5 (%15,6)
CERBB2 (n(%))	
(+)	7 (%21,9)
(−)	25 (%78,1)
Kİ 67 (n(%))	
(+)	25 (%78,1)
(−)	7 (%21,9)
EMG (n(%))	
Normal	30 (%93,8)
Sensory neuropathy	2 (%6,2)
LANSS score (n(%))	
0–12	28(87,5).
Above 12	4 (%12,5).

Table 4
Onconeuronal antibody results of the cases included in the study.

Antibody	
(+)	12 (%37,5)
(−)	20 (%62,5)
Amphipysin	
(+)	2 (%6,2)
(−)	30 (%93,8)
CV2	
(+)	4 (%12,5)
(−)	28 (87,5)
PNMA2Ma2Ta	
(+)	0
(−)	32 (%100)
Ri	
(+)	0
(−)	32 (%100)
Yo	
(+)	2 (%6,2)
(−)	30 (%93,8)
Hu	
(+)	2 (%6,2)
(−)	30 (%93,8)
Recoverin	
(+)	9 (%28,1)
(−)	23 (71,9)
SOX1	
(+)	0
(−)	32 (%100)
Titin	
(+)	4 (%12,5)
(−)	28 (87,5)
Zic4	
(+)	0
(−)	32 (%100)
GAD65	
(+)	0
(−)	32 (%100)
TrDNER	
(+)	0
(−)	32 (%100)

Table 5
Comparison of Tumor Grade and Antibody Positivity in Cases Involved in the Study.

	Antibody(−)	Antibody (+)	Total	p value
Tumor Grade				
Grade 1–2	13 (%86,7)	2 (%13,3)	15 (%100)	**0,008****
Grade 3	7 (%41,2)	10 (%58,8)	17 (%100)	
** Pearson chi-square test				

cases in which the cancer was not shown initially developed cancer within an average of 2 years [9]. Undoubtfully the presence of onconeural antibodies is a guide for paraneoplastic syndromes. However patients may contain high levels of antibodies without any symptoms in the nervous system [12]. Therefore, the relationship between antibodies and cancer is more pronounced than the relationship between neurological symptoms and onconeural antibodies. Onconeural antibodies are very rare in healthy individuals. In a study of blood samples taken from a high number of healthy adults, onconeural antibodies were observed only in less than 1% [13].

The presence of antibodies is also expected to be high during the period when tumor density is highest. Therefore, we aimed to show the relationship between parameters such as immunohistochemical markers, tumor grade and presence of autoantibodies at the time when tumor density was highest (before neoadjuvant chemotherapy) in patients with newly diagnosed breast cancer.

We detected neuropathy in 2 cases clinically and electrophysiologically. Prevalence of neuropathy appears to be low in breast cancer patients and therefore routine screening for peripheral nerve involvement is not recommended in breast cancer patients without neurological symptoms. One of these patients had CV2 antibody and EMG showed sensory neuropathy. In the other case, similar electrophysiological findings were found and Yo antibody was positive. In addition, the tumor grade was higher and the LANNS scale was above 12 in both patients. There was no significant relationship between hormone receptors (progesterone, estrogen), immunohistochemical markers such as Cerb2, Ki67, and perineural invasion that may be associated with pathogenesis in terms of diagnosis and prognosis of breast cancer patients. However, there is a correlation between the tumor

Table 6
Characteristics of two cases with sensory neuropathy.

	1.CASE	2.CASE
Age	56	54
Estrogen receptor	(+)	(+)
Progesterone receptor	(+)	(+)
C-erbB2	(−)	(−)
Ki-67	(−)	(+)
Tumor grade	3	3
Lanss score	15	15
Perineural invasion	(−)	(+)
EMG	Sensory Pnp	Sensory Pnp
Antibody positivity	CV2(+)	Yo (+)

grade and the positivity of onconeural antibodies. In patients presenting with neuropathic pain, if there is no significant disease that may cause underlying neuropathy, it is very important to think of possible malignancies and pathology findings such as tumor grade, which should be examined in terms of treatment and prognosis.

Although paraneoplastic neuropathies which are very rare in breast cancer-associated PNS, are frequently associated with CV2 and Hu antibodies it should be remembered that sensory neuropathy can be seen in the presence of non-neuropathy specific antibodies just as in our patient with Yo antibody.

On the other hand onconeural antibody positivity was observed in 37.5% of the cases included in the study. It is known that 60–70% of PNS detected in breast cancer patients have antibodies. In a recent study of 56 patients with PNS due to breast cancer, the antibody rate was 53.6% [14]. Our study has shown that antibody positivity is relatively high even in breast cancer patients without neurological symptoms, indicating that onconeural antibody positivity is not a specific and sensitive measure of PNS in breast cancer.

Many tumor antigens have been identified according to each breast cancer subtype. Mutation or deficiency in the p53 tumor suppressor gene is the most common cause of breast cancer. These p53 defects allow expression of mutated or misfolded proteins that are not visible to the immune system. Also hereditary and acquired defects in DNA repair may cause breast cancer. In addition many cellular mechanisms cause antigen expression. Due to certain cellular changes in the cancer cell and stroma, the immune system is designed to recognize tumor-associated epitopes as foreign. The microenvironment of the tumor includes immune cells such as CD4 + T, CD8 + T, Natural killer cells, macrophages, dendritic cells. Tumor cells are transported to lymph nodes or lymphatic organs to present antigen to CD4 + T, CD8 + T and B cells after apoptosis. Immune response secondary to enlargement of lymph nodes and organs with increased B and T lymphocytes occurs [15–19]. However B and T lymphocyte responses do not always produce an antitumoral response and sometimes are thought to cause autoantigen formation and autoimmunity triggering and/or paraneoplastic syndromes.

Onconeural antibodies are produced as an immune response to a tumor that ectopically expresses a neuronal antigen. These antibodies are then directed to antigens in the central and/or peripheral nervous systems. There is no evidence to support the general use of onconeural antibodies as potential cancer markers in individuals without neurological symptoms [9]. In clinical practice, it would be more beneficial to have antibody analysis limited to patients with neurological symptoms suspected of PNS.

8. Conclusions and recommendations

The response to treatment in paraneopastic neuropathies is limited. Therefore, biochemical markers should be developed for early diagnosis and treatment. Especially, suspicion of PNS is the most important step for clinicians in management of PNS. Overall, our results suggest that onconeural antibody positivity is not associated with presence of neuropathy and histochemical features of the tumor. However, detection of antibody appears to be an indicator of higher tumor grade and might thus be utilized as a marker of tumor prognosis or unfavorable outcome.

The incidence of cancer is increasing due to many reasons such as increased exposure to industrial toxins and at the same time prolonging survival depends on the success of the treatments. Despite the positive developments in the against cancer in recent years, the pathophysiology of cancer - related neuropathy is still unknown. The latest advances in technology, interdisciplinary neuroscience studies and the explanation of etiopathogenesis at molecular level will pave the way for the development of new biomarkers and new treatment methods.

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Conflicts of interest

The authors declares no conflicts of interest or disclosure associated with this publication.

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Concept - CS, HT; Design - CS, HT; Supervision – ET, MGG; Resource – MGG, FAV; Materials – ET, CS; Data Collection and/or Processing – CS, MGG, FAV; Analysis and/or Interpretation – HT, ET; Literature Search – CS; Writing – CS, ET; Critical Reviews – HT, MGG.

References

[1] R. Höftberger, M.R. Rosenfeld, J. Dalmau, Update on neurological paraneoplastic syndromes, Curr Opin Oncol 27 (6) (2015) 489.
[2] E. Tüzün, J. Dalmau, Limbic encephalitis and variants: classification, diagnosis and treatment, Neurologist 13 (5) (2007) 261–271.
[3] F. Leypoldt, K.P. Wandinger, Paraneoplastic neurological syndromes, Clinical & experimental immunology 175 (3) (2014) 336–348.
[4] F. Graus, J. Dalmau, Paraneoplastic neurological syndromes: diagnosis and treatment, Curr Opin Neurol 20 (6) (2007) 732–737.
[5] M.A. Kanikannan, et al., Incidence and spectrum of paraneoplastic neurological syndromes: single center study, J Neurooncol 125 (1) (2015) 197–206.
[6] C. Vedeler, et al., Management of paraneoplastic neurological syndromes: report of an EFNS task force, Eur J Neurol 13 (7) (2006) 682–690.
[7] S. Koçak, et al., Meme kanserinde risk faktörleri, riskin değerlendirilmesi ve prevansiyon: İstanbul 2010 konsensus raporu, Meme Sagligi Dergisi/Journal of Breast Health 7 (2011) 2.
[8] T.B. Tarr, P. Wipf, S.D. Meriney, Synaptic pathophysiology and treatment of Lambert-Eaton myasthenic syndrome, Mol Neurobiol 52 (1) (2015) 456–463.
[9] C.D. Savci-Heijink, et al., Retrospective analysis of metastatic behaviour of breast cancer subtypes, Breast Cancer Res Treat 150 (3) (2015) 547–557.
[10] J.-C. Antoine, J.-P. Camdessanché, Peripheral nervous system involvement in patients with cancer, The Lancet Neurology 6 (1) (2007) 75–86.
[11] H. Tankisi, et al., Pathophysiology inferred from electrodiagnostic nerve tests and classification of polyneuropathies. Suggested guidelines, Clin Neurophysiol 116 (7) (2005) 1571–1580.
[12] J. Honnorat, A. Viaccoz, New concepts in paraneoplastic neurological syndromes, Rev Neurol 167 (10) (2011) 729–736.
[13] S.J. Pittock, T.J. Kryzer, V.A. Lennon, Paraneoplastic antibodies coexist and predict cancer, not neurological syndrome, Ann Neurol 56 (5) (2004) 715–719.
[14] S.E. Monstad, et al., Onconeural antibodies in sera from patients with various types of tumours, Cancer Immunol Immunother 58 (11) (2009) 1795–1800.
[15] B.L. Murphy, et al., Breast cancer-related paraneoplastic neurologic disease, Breast Cancer Res Treat 167 (3) (2018) 771–778.
[16] I. Fanous, P. Dillon, Paraneoplastic neurological complications of breast cancer, Exp Hematol Oncol 5 (1) (2015) 29.
[17] H.T. Tan, et al., Serum autoantibodies as biomarkers for early cancer detection, FEBS J 276 (23) (2009) 6880–6904.
[18] E.M. Tan, Autoantibodies as reporters identifying aberrant cellular mechanisms in tumorigenesis, J Clin Invest 108 (10) (2001) 1411–1415.
[19] H. Lu, V. Goodell, M.L. Disis, Humoral immunity directed against tumor-associated antigens as potential biomarkers for the early diagnosis of cancer, J Proteome Res 7 (4) (2008) 1388–1394.