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MAJOR ARTICLE

Monocyte Gene Expression Distinguishes Enhancing Brain Parenchymal Cysticercal Granulomas From Tuberculomas

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Background. In patients with enhancing brain parenchymal lesions, parenchymal neurocysticercosis (pNCC) is often difficult to distinguish from tuberculoma, necessitating biopsy or empirical therapy.

Methods. In a prospective study, peripheral blood monocytes were isolated from patients with definitive pNCC (n = 39) and brain tuberculomas (n = 20). Patients with tuberculomas were diagnosed by the presence of concurrent systemic tuberculosis (n = 7), pathological or bacteriological confirmation (n = 5), and resolution of typical brain lesions following a therapeutic trial of antituberculous therapy (n = 8). Expressions of 14 NCC-associated monocyte genes were determined by quantitative polymerase chain reaction and analyzed for diagnostic usefulness between the 2 groups.

Results. Expression of 7 genes (TAX1BP1, RAP1A, PLCG2, TOR3A, GBP1P1, LRRFIP2, and FEZ2) was significantly higher in pNCC patients than in tuberculoma patients, with TAX1BP1 and RAP1A expressions more than 22- and 5-fold higher in pNCC patients. TAX1BP1 had the highest sensitivity of 66.7% at a specificity of 100% in discriminating pNCC from tuberculoma. A combination of TAX1BP1 and RAP1A increased the sensitivity to 84.6%, and including GBP1P1 with TAX1BP1 and RAP1A further increased sensitivity to 87.2% while maintaining specificity of 100%.

Conclusions. Expression of a panel of genes in blood monocytes distinguishes pNCC from brain tuberculomas in patients with enhancing brain lesions.

Keywords. brain tuberculoma; enhancing brain lesion; monocyte genes; neurocysticercosis.

In regions of the world endemic for cysticercosis and tuberculosis such as South Asia, China, Sub-Saharan Africa, and Latin America, small, enhancing lesions in the brain, either solitary or multiple, can pose a diagnostic challenge. The differential diagnosis of such lesions typically includes cysticercal granulomas and tuberculomas [1]. A solitary cysticercal granuloma (SCG), the degenerative granular nodular stage of the parasite, can often be distinguished from a solitary small tuberculoma using validated diagnostic criteria [2, 3]. Nevertheless, the diagnosis is only confirmed after several months of clinical and imaging follow-up [4, 5]. Moreover, the imaging features of an SCG can be atypical, in which case there could be doubts about the diagnosis (Figure 1A–C). In addition, it can be difficult to differentiate parenchymal neurocysticerosis (pNCC) that presents on magnetic resonance imaging (MRI) purely as multiple enhancing granulomas, without calcific lesions or live cysts with scolices, from multiple small tuberculomas without resorting to a biopsy (Figure 1D–G) [4–7]. Patients with brain tuberculomas present with raised intracranial pressure, focal neurological deficits, or seizures, like any other intracranial mass lesion. Constitutional symptoms of tuberculosis such as fever, sweats, and weight loss are not usually seen in patients with isolated brain tuberculomas without tuberculous meningitis [8]. Brain tuberculomas are often diagnosed on the basis of imaging findings, along with response to empiric therapy. But in some instances, diagnosing brain tuberculoma in the absence of synchronous systemic tuberculosis is difficult and requires histological confirmation [9]. Tuberculin skin test and interferon-gamma release assay (IGRA) in patients with central nervous system tuberculosis and brain tuberculomas have low sensitivity [10]. Moreover, the tuberculin skin test is often positive in asymptomatic people in India due to mucosal exposure to *Mycobacterium tuberculosis* in the environment [11].

When a clear distinction between pNCC and tuberculoma cannot be made on imaging, brain biopsy is often the only option available to establish diagnosis. However, in most clinical

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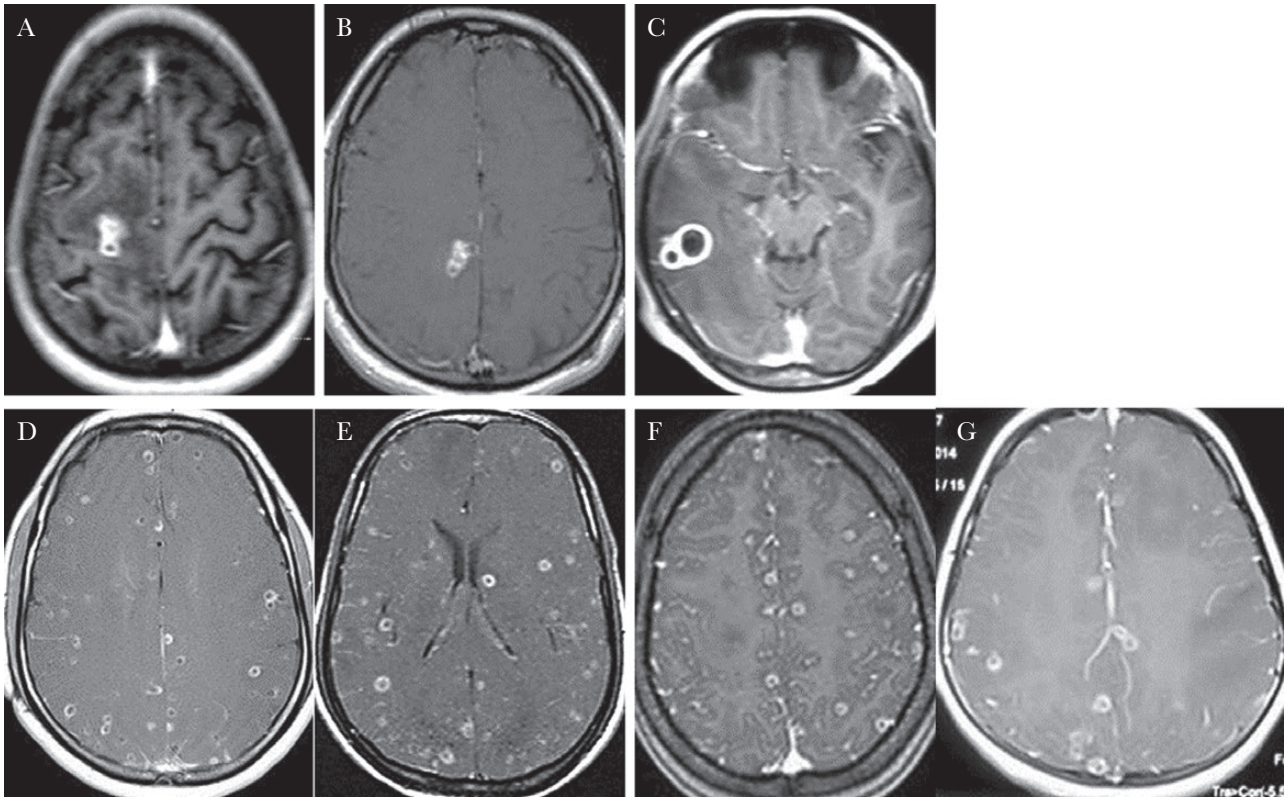

Figure 1. MR images of atypical solitary cysticercus granulomas and multiple parenchymal cysticercal granulomas and tuberculomas. A–C, T1W gadolinium–enhanced MR images showing atypical appearances of solitary cysticercus granuloma in 3 patients. D–G, Representative T1W axial postgadolinium MR images illustrating the diagnostic difficulty in patients with multiple small enhancing brain lesions. D and E, MR images from 2 patients with parenchymal neurocysticercosis; diagnosis was made on the basis of presence of live cysts/calcified NCC (seen in other sections not shown here) and positive serological tests for NCC. F and G, MR images from 2 patients with brain tuberculomas; diagnosis was made on the basis of synchronous systemic tuberculosis. Abbreviations: MR, magnetic resonance; NCC, neurocysticercosis.

settings, clinicians resort to empiric treatment with a therapeutic trial of albendazole or antituberculous therapy, and may change from 1 treatment to the other when there is no clinical or radiological response [12].

Serological tests for cysticercosis such as enzyme-linked immunoelectrotransfer blot (EITB) for cysticercal antibodies and antigen enzyme-linked immunosorbent assay (ELISA) have low sensitivity in patients with SCG. These tests can be positive in patients with brain tuberculomas residing in regions endemic for cysticercosis due to their exposure to the larval antigen or due to cysts located in peripheral tissues [13–16]. It is possible that an individual who has a brain tuberculoma might be sero-positive on EITB due to exposure to the larval antigens and would be incorrectly diagnosed to have NCC if EITB was used as the sole distinguishing test. Thus, it is desirable to develop a noninvasive test that distinguishes pNCC from brain tuberculoma in patients with inconclusive brain imaging to obviate the need for a diagnostic brain biopsy.

In an earlier study [17] that used microarray analysis of mRNA expression in blood monocytes from patients with pNCC (n = 6), epilepsy of unknown etiology (EUE; n = 6), and headache control (n = 4), 14 genes were noted to be upregulated and significantly associated with pNCC. These 14 genes, selected from among 1411 upregulated genes, were associated with helminth infections, inflammation, and neurological disorders based on the open source database of PUBMED and PANTHER software, version 10 [17]. The 14 genes were GTP-related (CHN2, GBP1, GBP1P1, PLCG2, RAP1A, and TAGAP), immune-related (IL20RB, LRRFIP2, and TAX1BP1), those involved with neural processes (FEZ2 and TOR3A), and miscellaneous genes (MZB1, SLC8A1, and PECAM1). The aim of the present study was to determine if expression of these 14 monocyte genes can aid in differentiating pNCC and brain tuberculomas in patients presenting with enhancing lesions on brain imaging.

METHODS

Patient Consent

The study was approved by the Institutional Review Boards of the Christian Medical College, Vellore, India (IRB Min. No. 10130) and of the University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA (IRB Min. No. 6831).

Informed written consent was obtained from all individuals recruited into the study.

Patients

All patients included in the study were aged between 18 and 51 years and presented to the Department of Neurological Sciences, Christian Medical College, Vellore, India, for the first time between May 2017 and November 2019. All patients had either a brain computed tomography (CT) scan, MRI, or both. All patients were tested for serum cysticercus antibodies by EITB and for circulating cysticercal antigens by an antigen ELISA [13]. All consecutive patients who presented to the outpatient clinic with clinical and radiological findings of an intraparenchymal granuloma during the study period were screened and included if they gave consent.

The diagnosis of pNCC and tuberculoma were based on a combination of clinical, histopathological, microbiological, and radiological criteria and were used as the gold standard. Preliminary studies to establish diagnostic tests require using samples that are well defined and satisfy gold standard criteria.

Parenchymal NCC

Thirty-nine patients with definitive pNCC were included in the study; 22 patients had SCG, and 17 had multiple lesions. All 22 patients with SCG fulfilled the validated diagnostic criteria [3]. All 17 patients with multiple lesions fulfilled the published criteria for diagnosis of NCC by Del Brutto et al. and Carpio et al. [5, 18]. The relevant diagnostic criteria utilized in this study for diagnosis of definitive pNCC are summarized (Table 1). All patients with pNCC presented with seizures (median duration [interquartile range {IQR}], 7 [1–39] months) and had at least 1 seizure (range of total number of seizures, 1–20) in the 7 months before blood collection. All pNCC (22 SCGs and 17 with multiple lesions) patients had enhancing lesions <20 mm in size.

Brain Tuberculomas

Twenty patients with brain tuberculomas were included in the study. Seizure(s) was the presenting symptom in 7 patients (median duration [IQR], 4.5 [3–8] months). The remaining 13 patients presented with features of raised intracranial pressure and/or progressive neurological deficits (median duration of symptoms [IQR], 4 [2.5–6] months). Constitutional symptoms such as fever or weight loss were present in 4 patients, all of whom had synchronous systemic tuberculosis. None of the patients with isolated brain tuberculomas without systemic disease (n = 8) had constitutional symptoms. Tuberculin skin testing and the IGRA were not performed in our patients. Radiological diagnosis of brain tuberculoma was based on the characteristic findings seen on contrast-enhanced MRI (markedly hypointense lesions on T2W sequences, thick enhancing wall of the lesion, solitary or conglomerate lesions, enhancing lesions within the subarachnoid space, or presence of basal exudates) [8].

Diagnosis of tuberculoma was histologically and/or bacteriologically confirmed in 12 patients. A positive culture for acid fast bacilli was obtained in 8 of these 12 patients.

In the 8 patients who were treated empirically with antituberculous therapy on the basis of MRI findings typical of tuberculoma, the diagnosis was confirmed by radiological resolution, either complete (4) or partial (4), and clinical improvement with antituberculous therapy at a follow-up (range) of ≥3

Table 1. Summary of Diagnostic Criteria for pNCC (adapted from Rajshekhar et al. [3]; Del Brutto et al. [5]; Carpio et al. [18]) and its Application to our Patients With pNCC (n = 39)

Criterion	Description
Absolute	1. Conclusive demonstration of scolex within a cystic lesion on neuroimaging studies [5, 18]
Neuroimaging (major)	1. Cystic lesions without a discernible scolex (*well-defined, rounded lesions with CSF signal intensity on CT/MRI*) [5, 18] 2. Enhancing lesions <20 mm in diameter within brain parenchyma (*single or multiple ring or nodular enhancing lesions with or without perilesional edema, but not displacing midline structures*) [4, 5, 18] 3. Typical brain parenchymal calcifications (*single or multiple solid lesions, usually <10 mm in diameter*) [5]
Neuroimaging (confirmatory)	1. Spontaneous resolution of single small enhancing lesions (*corticosteroid therapy precludes use of this criterion*) [5] 2. Resolution of cystic lesions after cysticidal drug therapy [5]
Clinical/exposure (major)	1. Detection of anticysticercal antibodies (EITB) or cysticercal antigens (antigen ELISA) by well-standardized immunodiagnostic tests [5, 18] 2. Evidence of household contact with *T. solium* infection [5]
Clinical/exposure (minor)	1. Clinical manifestations suggestive of neurocysticercosis—seizures [3–5, 18] 2. Individuals coming from or living in an area where cysticercosis is endemic [5]
Definitive diagnosis of pNCC	Presence of any of the following [5]: A. 1 absolute criterion B. 2 major neuroimaging criteria + any clinical/exposure criteria C. 1 major neuroimaging criterion + 1 confirmative neuroimaging criterion + any clinical/exposure criteria D. One major neuroimaging + any 2 clinical/exposure criteria (including 1 major clinical/exposure criterion) E. Any combination of parenchymal cysts in different stages of evolution—vesicular (with or without scolex), degenerative (colloidal or vesicular), and calcified [18]

Abbreviations: CSF, cerebrospinal fluid; CT, computed tomography; EITB, enzyme-linked immunoelectrotransfer blot; ELISA, enzyme-linked immunosorbent assay; MRI, magnetic resonance imaging; pNCC, parenchymal neurocysticercosis; SCG, solitary cysticercal granuloma.

(3–18) months. All 8 patients had enhancing lesions, and none had imaging features suggestive of NCC. Details of diagnosis of brain tuberculomas are summarized (Table 2).

Diagnosis and classification of brain lesions were performed by the senior author (V.R.) before quantitative polymerase chain reaction (qPCR) assays.

Peripheral Blood Monocyte Isolation and qPCR of Monocyte Genes

Twelve milliliters of peripheral blood was collected in EDTA tubes, and CD14+ monocytes were isolated as described previously [17]. RNA was extracted from monocytes with TRI Reagent following the manufacturer's instructions (Sigma-Aldrich Chemical Co, St. Louis, MO, USA); 0.5 µgm of RNA was treated with DNase (Ambion Life Technologies Co, Waltham, MA, USA) and reverse-transcribed to cDNA (as described by Invitrogen Life Technologies Co, Waltham, MA, USA). cDNA from all participants was subject to qPCR (Quant Studio 5) for the 14 genes studied earlier using SYBR PCR Master Mix (Applied Biosystems, Waltham, MA, USA) and consensus primers (synthesized at Sigma-Aldrich Chemicals Pvt Ltd, Bangalore, India) [17]. The genes analyzed were CHN2, GBP1, GBP1P1, PLCG2, RAP1A, TAGAP, IL20RB, LRRFIP2, TAX1BP1, FEZ2, TOR3A, MZB1, SLC8A1, and PECAM1. The housekeeping gene was β2-microglobulin (β2-M). Gene expression was determined by normalization to the housekeeping gene β2-M and calculated as $2^{-\Delta Ct}$ where ΔCt = Ct gene – Ct β2M [17, 19]. Fold change gene expression between the 2 patient groups was calculated for each gene as mean of $2^{-\Delta Ct}$ pNCC/mean of $2^{-\Delta Ct}$ tuberculoma. qPCR assays were carried out by laboratory personnel blinded to the clinical status of the patient.

Statistical Analysis

The characteristics between the 2 groups were compared and analyzed using the chi-square and Mann-Whitney *U* tests. Normalized gene expression was entered into an Excel spreadsheet. The mean and SD of the normalized expression of each gene in the pNCC and tuberculoma groups were calculated. The Mann-Whitney *U* test was used to compare gene expressions between the 2 groups for significant differences ($P < .05$). Receiver operating characteristic (ROC) analysis was performed using MedCalc Software (version 5.8) to estimate the sensitivity, specificity, and gene expression cutoff value of each gene to classify samples as pNCC ("true positive") or tuberculoma ("true negative").

RESULTS

Patient Characteristics

The median age of the patients in the pNCC and tuberculoma groups was similar. There were significantly more males in the pNCC group compared with the brain tuberculoma group. The proportion of solitary lesions in the pNCC group was significantly higher. Serum cysticercal antibodies (EITB) were positive in 27 of 39 (69.2%) pNCC patients and in 1 of 20 (5%) tuberculoma patients. Antigen ELISA was positive in 17 of 39 (43.6%) pNCC patients and in none of the tuberculoma patients. Thirteen of 39 patients with pNCC tested positive for cysticercal antibodies and antigens. Thirty-one of the 39 patients were positive for either cysticercal antibodies or antigens (Table 3).

Monocyte Gene Expression in pNCC and Tuberculoma Patients

The expressions of 7 genes (TAX1BP1, RAP1A, GBP1P1, PLCG2, TOR3A, LRRFIP2, and FEZ2) were significantly higher in the pNCC than the tuberculoma group, ranging between 4- and 22-fold (Table 4).

Receiver Operating Characteristic Analysis

The sensitivity and specificity of each gene in distinguishing pNCC from tuberculoma and the gene expression threshold value for maximum sensitivity at a specificity of 100% are reported (Table 5).

At a specificity of 100%, TAX1BP1 demonstrated the highest sensitivity, 66.7%, for pNCC with an ROC cutoff threshold expression $2^{-\Delta Ct}$ value of 0.018, followed by RAP1A and GBP1P1 with cutoff threshold expression values/sensitivities of 0.033/46.2% and 0.017/20.5%, respectively. The ROC curves of these 3 genes are presented in Figure 2.

The sensitivity in distinguishing pNCC from tuberculoma in the study patients increased to 87.2% when expression of at least 1 of the following 3 genes, TAX1BP1, RAP1A, and GBP1P1, was

Table 2. Diagnosis of Brain Tuberculoma (n = 20)

Mode of Diagnosis	No. of Patients
M. tuberculosis demonstrated in brain or systemic lesion	
Brain lesion/cerebrospinal fluid	
Synchronous systemic tuberculosis	5
(Lymph node [n = 1]; sputum [n = 1]; bone marrow [n = 1])	3
Necrotizing granulomatous inflammation on histopathology of brain/systemic lesion	4
Typical MRI findings and resolution of clinical symptoms and MR lesions with antituberculous therapy *Presence of tuberculous meningitis (n = 2)*	8

Abbreviations: MR, magnetic resonance; MRI, magnetic resonance imaging.

Table 3. Patient Characteristics

Characteristic	Neurocysticercosis (n = 39)	Tuberculoma (n = 20)	P Value
Demographics			
Male: female	34:5	11:9	.01
Median age (IQR), y	29 (22–32.5)	28 (23–38.3)	.69
Image feature			
Solitary lesion/conglomerate lesions in single location	22	5	.03
Multiple lesions	17	15	
Scolex present	SCG—4 (18.2%) Multiple lesions—9 (52.9%)	0	
Medications			
Exposure to antituberculous therapy (before or at time of blood collection)	4	15	<.00001
Albendazole therapy received	12	2	.08
Corticosteroid treatment within 7 d of blood collection	0	10	
Serological tests			
EITB positive	27	1	
Antigen ELISA positive	17	0	
Positive for EITB and antigen ELISA	13	0	

Abbreviations: EITB, enzyme-linked immunoelectrotransfer blot; ELISA, enzyme-linked immunosorbent assay; IQR, interquartile range; SCG, solitary cysticercal granuloma.

above its ROC cutoff threshold expression value while the specificity remained at 100% when all genes were below these values (Table 6).

The expression of all 3 genes (TAX1BP1, RAPIA, and GBP1P1) was similar in the patients with tuberculoma who received corticosteroids (n = 10) vs those who did not receive corticosteroids (n = 10) (Supplementary Table 1).

The fold change gene expressions between the confirmed tuberculoma (n = 12) and empirically treated tuberculoma (n = 8) groups were similar for the panel of 3 genes, TAX1BP1, RAPIA, and GBP1P1 (Supplementary Table 2). ROC analysis of the combination of the 3 genes to distinguish pNCC (n = 39) from empirically treated TB (n = 8) and confirmed tuberculoma (n = 12) maintained the sensitivity at 89% and 87% at 100% specificity, respectively (Supplementary Tables 3 and 4).

The gene expression levels and sensitivity of the test remained the same regardless of the number of lesions.

DISCUSSION

Differentiating pNCC From Tuberculoma on Brain Imaging

It is important to distinguish between pNCC and tuberculoma as their management is different [4, 9, 20]. SCGs can be diagnosed using validated diagnostic criteria with sensitivity and specificity of >98% [2, 3]. Based on these criteria, lesion size

Table 4. Monocyte Gene Expression, Fold Change, and P Value of the Mann-Whitney Test Comparing pNCC and Tuberculoma for 14 Genes

Gene	pNCC (n = 39) $2^{(-Ct\ gene - Ct\ \beta2M)}$ Mean ± SD	TB (n = 20) $2^{(-Ct\ gene - Ct\ \beta2M)}$ Mean ± SD	Fold Change of Mean $2^{(-Ct\ gene - Ct\ \beta2M)}$ pNCC/TB	P Value (Mann-Whitney Test)
TAX1BP1	0.139 ± 0.466	0.006 ± 0.005	22.3	<.0001
RAP1A	0.051 ± 0.078	0.009 ± 0.009	5.2	.004
GBP1P1	0.046 ± 0.142	0.003 ± 0.004	12.2	.02
PLCG2	0.329 ± 1.118	0.019 ± 0.025	17.2	.01
TOR3A	0.070 ± 0.190	0.016 ± 0.041	4.3	.01
LRRFIP2	0.219 ± 0.645	0.025 ± 0.062	8.7	.02
FEZ2	0.070 ± 0.148	0.013 ± 0.033	5.1	.02
MZB1	0.056 ± 0.098	0.017 ± 0.019	3.2	.1
PECAM1	0.075 ± 0.116	0.027 ± 0.023	2.8	.1
SLC8A1	0.605 ± 1.054	0.287 ± 0.328	2.1	.2
CHN2	0.255 ± 0.599	0.028 ± 0.030	9.1	.2
TAGAP	0.275 ± 1.066	0.220 ± 0.347	1.3	.5
GBP1	0.086 ± 0.165	0.048 ± 0.066	1.8	.7
IL20RB	0.038 ± 0.146	0.007 ± 0.015	4.8	.8

Abbreviations: pNCC, parenchymal neurocysticercosis; TB, tuberculoma.

Table 5. Receiver Operating Characteristic Analysis, P Value, Cutoff Using $2^{-\Delta Ct}$ and Sensitivity at 100% Specificity for 14 Genes to Discriminate pNCC (n = 39) From Tuberculoma (n = 20)

S. No.	Gene	Area Under Curve	95% CI	P Value	Cutoff $2^{-Ct\ gene - Ct\ \beta 2M}$	Sensitivity, %
1	TAX1BP1	0.899	0.793–0.962	<.0001	>0.0187	66.7
2	RAP1A	0.733	0.601–0.840	.0003	>0.033	46.2
3	GBP1P1	0.688	0.554–0.802	.008	>0.0172	20.5
4	PLCG2	0.703	0.570–0.815	.004	>0.1023	25.6
5	TOR3A	0.699	0.566–0.812	.005	>0.1873	10.3
6	LRRFIP2	0.687	0.553–0.802	.01	>0.2802	10.3
7	FEZ2	0.687	0.553–0.801	.008	>0.1551	10.3
8	MZB1	0.632	0.496–0.754	.08	>0.0774	12.8
9	PECAM1	0.621	0.485–0.744	.1	>0.0824	25.6
10	SLC8A1	0.604	0.469–0.729	.2	>0.9196	23.1
11	CHN2	0.596	0.460–0.722	.2	>0.1069	23.1
12	TAGAP	0.560	0.424–0.689	.5	>1.421	0
13	GBP1	0.537	0.393–0.658	.7	>0.224	12.8
14	IL20RB	0.519	0.385–0.651	.8	>0.0689	7.7

Abbreviation: pNCC, parenchymal neurocysticercosis.

>20 mm excludes the diagnosis of cysticercal granuloma. However, the presence of multiple lesions, all <20 mm in size, can make it difficult to distinguish between pNCC and tuberculoma, as well as from other pathologies such as metastasis or pyogenic/fungal abscesses [12, 21–23]. Multiple pNCC is diagnosed with near certainty on MRI when scolices are identified in any of the lesions or when there are lesions displaying different stages of the cysticercus, that is, live cysts, granulomas, and calcifications. But it is difficult to make a diagnosis with certainty when only enhancing parenchymal lesions are seen [6, 12, 21–23]. In a series of 110 Indian patients with multiple brain lesions, tuberculosis and cysticercosis were the most common diagnoses [6]. In this series, it was necessary to initiate empiric antituberculous therapy in 45 patients despite a battery of investigations. Upon follow-up of these patients, pulmonary metastases were detected in 6 patients, whereas brain lesions resolved partially or totally in 11 patients but persisted in 24 patients despite antituberculous therapy [6].

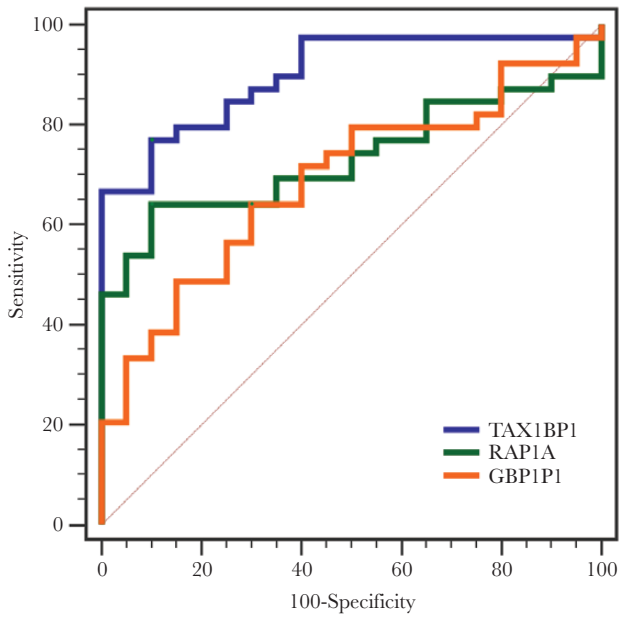

Figure 2. Receiver operating characteristic curves for monocyte genes in pNCC compared with tuberculoma. At maximum sensitivity and corresponding specificity, the area under the curve for TAX1BP1 was 0.899 ($P < .001$), for RAP1A it was 0.733 ($P = .0003$), and for GBP1P1 it was 0.688 ($P = .008$). At a specificity of 100%, the sensitivity of TAX1BP1 was 66.7%, RAP1A 46.2%, and GBP1P1 20.5% in distinguishing pNCC from tuberculoma in patients with enhancing brain lesions. Abbreviation: pNCC, parenchymal neurocysticercosis.

Although studies report that specialized imaging sequences on MRI might differentiate pNCC from tuberculomas, several patients recruited in these studies did not have a definitive diagnosis for either pathology. Parry et al. [24] reported that the presence of a complete hypointense ring on susceptibility weighted imaging (SWI) may favor diagnosis of tuberculoma over metastasis, glioma, or pNCC. Two of the 6 patients in this series with NCC had a complete hypointense ring, rendering this finding a poor discriminator between tuberculoma and NCC. Moreover, only 4 of 72 patients with "tuberculomas" in this study had histopathological confirmation, and no other imaging findings were specific to tuberculomas.

In a study on solitary enhancing lesions, Pretell et al. [25] reported that tuberculomas (n = 4, diagnosed by imaging and negative serology for NCC) exhibit lipid peak on magnetic resonance spectroscopy (MRS), while NCC lesions (n = 6, all EITB positive) do not. As 30%–40% of patients with SCG have a negative EITB, this criterion might have led the authors to misdiagnose some SCGs as tuberculoma [14]. Two of their patients with "tuberculomas" had solitary lesions <2 cm in size. Moreover, MRS becomes difficult to interpret in lesions measuring <2 cm as the voxel tends to include adjacent

Table 6. Sensitivity and Specificity of a Combination of TAX1B1P1, RAP1A, and GBPIP1 to Distinguish pNCC (n = 39) From Tuberculoma (n = 20) Cases

	Parenchymal Neurocysticercosis (n = 39)			Tuberculoma (n = 20)		
Gene Combination	Positive, No.	Negative, No.	Sensitivity (95% CI), %	Positive, No.	Negative, No.	Specificity (95% CI), %
TAX1BP1	26	13	66.7 (49.8–80.0)	0	20	100 (83.2–100)
TAX1BP1 + RAP1A	33	6	84.6 (69.5–94.1)	0	20	100 (83.2–100)
TAXIBP1 + RAPIA + GBP1P1	34	5	87.2 (72.6–96)	0	20	100 (83.2–100)

Abbreviation: pNCC, parenchymal neurocysticercosis.

brain parenchyma not involved by the lesion, which decreases the reliability of this technique.

Ghosh et al. [26] observed significantly higher relative cerebral blood volume (rCBV values) in solitary tuberculomas (n = 20) than in solitary pNCC (n = 30) and reported cutoff values with a sensitivity of 90% and a specificity of 100%. However, several of their patients may not fulfill the validated diagnostic criteria for SCG.

In another study, vesicular and degenerating cysticercal lesions had significantly higher diffusion coefficient values than tuberculomas or tuberculous brain abscesses [27]. Measurement of rCBV and diffusion coefficient requires a trained radiologist for interpretation, and this may not be always possible in areas with limited resources.

Limitations of Serological Tests for pNCC

Serological tests for pNCC (EITB for cysticercal antibodies and antigen ELISA) could potentially be used to differentiate between pNCC and brain tuberculomas [13, 14]. However, up to 15.9% of asymptomatic individuals have been reported to be seropositive for cysticercal antibodies in endemic regions [28]. Additionally, EITB cannot be the sole test used to exclude a diagnosis of SCG, as the sensitivity of the test for solitary lesions is <70% [14, 15].

Circulating cysticercal antigens are associated with live cysts in the brain or cysticercosis elsewhere in the body. The sensitivity of the antigen ELISA is low in the absence of live cysts (granulomas are degenerating cysts), and a negative test would not reliably differentiate between NCC and tuberculoma [15, 16].

In our study, the serological tests for cysticercal antibody and antigen detection (EITB and Ag-ELISA respectively) showed a sensitivity of 79.5% (31/39) for pNCC with a specificity of 95% (Table 3). The tests thus did not diagnose 20% of pNCC patients, indicating the need for improved diagnostic accuracy for pNCC, especially in regions endemic for *T. solium,* where the prevalence of the infection is high.

Gene Expression in pNCC

We previously reported increased expression of 14 monocyte genes in pNCC patients compared with patients with idiopathic epilepsy or headaches [17]. In the present report, 7 of these genes had higher expression in monocytes of patients with definitive pNCC compared with patients with tuberculoma. The increased expression of any 1 of the 7 genes had a low sensitivity in discriminating pNCC from tuberculomas. However, a combination of 3 genes, TAX1BP1, RAP1A, and GBP1P1, increased the sensitivity for differentiating pNCC from tuberculomas to >87% from their individual values of 66%, 46%, and 20%, respectively. In the tuberculoma group, expressions of all 3 genes were consistently low despite the variability in size, number of lesions, and radiological and clinical presentations. Corticosteroid therapy had no influence on the expression of these 3 genes. Hence, with the 3-gene combination, specificity was maintained at 100% in excluding tuberculoma from pNCC. These findings improve on existing serological tests for NCC that have a sensitivity of 69.3% in patients with pNCC (86% in multiple and 62.6% in solitary lesions) with the additional limitations of interpreting the results of these tests noted above [15].

Our results suggest that a panel of the genes TAX1BP1, RAP1A, and GBP1P when expressed over an empirically determined expression threshold cutoff level could be useful in excluding the diagnosis of tuberculoma in patients for whom brain imaging cannot distinguish between NCC and tuberculoma. This would guide management and prevent exposure of patients with NCC to empirical antituberculous therapy.

In this preliminary study, relative levels of gene expression are analyzed. Consequently the gene expression threshold cutoff is a relative expression value that may differ between these patient groups in different centers. Quantification of mRNA would enable establishment of absolute gene expression threshold cutoff values that would be universally applicable and hence more useful.

The Roles of TAX1BP1, RAP1A, and GBP1P1 in Infections

Expression profiles of specific peripheral blood monocyte genes distinguish pNCC from brain tuberculoma with high sensitivity and specificity. TAX1BP1 is reported to be involved in pathways regulating autophagy, in activation of germinal center formation on stimulation of B cells, and in regulating T-lymphocyte responses [29, 30]. Budzik et al. [31] have recently shown that TAX1BP1 is

involved in the inflammatory response to *M. tuberculosis* infection and that TAX1BP1 deficiency results in impaired autophagosome maturation and clearance of ubiquinilated *M. tuberculosis* within macrophages. Augmented growth of *M. tuberculosis* was observed in *M. tuberculosis*–infected bone marrow–derived macrophages from TAX1BP1 knockout mice compared with wild-type mice [31]. Thus lower expression of TAX1BP1 may have a role in persistence of the bacteria and subsequent tuberculoma formation in the brain. This requires further investigation.

RAP1A and GBP1P1 are also involved in infection control, but their roles in the pathogenesis of NCC and tuberculoma need to be studied. RAP1A overexpression in macrophages protects parasites from phagocytosis, while in RAP1A knockout mice activation of phagocytosis through the FcγR receptor has been noted [32, 33]. There are reports to show that RAP1A is activated through calcium influx as a result of TLR signaling with trigger of innate immune responses [34, 35]. RAP1A is also involved in cAMP signaling and inositol triphosphate pathways, which regulate both innate and adaptive immune activities [36, 37]. GBP1P1 is a pseudogene that may be involved in regulating the parental gene expression of type 1 interferon (IFN1) and thus play a role in initiating innate immune responses that are involved with inflammasomes and autophagy [38, 39].

Future Directions

We have provided proof of concept for using the expression of a combination of genes in monocytes to distinguish pNCC from tuberculoma in patients with enhancing brain lesions. To quantify the threshold of gene expression, a nanostring approach may need to be undertaken. Our findings need to be validated in larger patient cohorts.

As monocyte isolation followed by RNA extraction and qPCR may not be an ideal workflow for a diagnostic test, other strategies will need to be explored to use our findings in a clinical setting. As a follow-up of this study, we are investigating next-generation sequencing in blood that could provide potential biomarkers (including these 3 genes) to distinguish pNCC from tuberculoma.

CONCLUSIONS

This study indicates the possibility of using peripheral blood monocyte genes to distinguish pNCC from brain tuberculoma with high sensitivity and specificity in patients with enhancing lesions on brain images. A panel of genes consisting of TAX1BP1, RAP1A, and GBP1P1 could be used in a qPCR test to exclude the diagnosis of tuberculoma in patients for whom pNCC and tuberculomas are the 2 main differential diagnoses.

Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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