ORIGINAL ARTICLE

Diagnostic and Prognostic Utility of Measuring Tumor Necrosis Factor in the Peripheral Circulation of Patients With Immune-Mediated Sensorineural Hearing Loss

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Objectives: To characterize levels of tumor necrosis factor (TNF; formerly known as tumor necrosis factor α), a well-established proinflammatory cytokine, in patients with immune-mediated sensorineural hearing loss (IM-SNHL) and to determine the role of this cytokine in identifying steroid-responsive hearing loss.

Design: Prospective case-control study.

Setting: Tertiary care academic medical center.

Patients: A total of 11 control subjects and 85 patients with clinical and audiometric characteristics of IM-SNHL (autoimmune inner ear disease and sudden SNHL combined) treated with corticosteroids were enrolled in the study. Patients were categorized as steroid responders (n=47) and steroid nonresponders (n=38). Peripheral venous blood was used to determine the total amount of plasma TNF by enzyme-linked immunosorbent assay. Peripheral blood mononuclear cells (PBMCs) were isolated and treated with in vitro dexamethasone. Treated and untreated PBMCs were then analyzed for release of soluble TNF protein into conditioned supernatants as well as expression of TNF messenger RNA (mRNA).

Main Outcome Measures: Mean plasma levels of TNF, unstimulated and dexamethasone-stimulated PBMC-secreted levels of TNF, and TNF mRNA levels in unstimulated and dexamethasone-stimulated PBMCs.

Results: Steroid nonresponders had the highest mean baseline plasma levels of TNF compared with steroid responders and control subjects (27.6, 24.1, and 14.4 pg/mL, respectively) ($P=.03$). For patients with IM-SNHL with a high baseline plasma levels of TNF (>14.4 pg/mL), the mean TNF secreted by PBMCs was 59.1 pg/mL, which decreased to 7.2 pg/mL with in vitro dexamethasone stimulation in the responder group, while the mean TNF secreted by PBMCs was 11.2 pg/mL, which slightly increased to 11.7 pg/mL with in vitro dexamethasone stimulation in the nonresponder group ($P=.04$).

Conclusions: The level of TNF can be used as both a diagnostic and prognostic cytokine for IM-SNHL. For patients presenting with a sudden change in hearing threshold, a high baseline plasma TNF from the peripheral circulation is supportive of the diagnosis if it is greater than 18.8 pg/mL, with a positive predictive value higher than 97%. In addition, this study demonstrates that for patients with IM-SNHL and high plasma levels of TNF, their clinical response to oral glucocorticoids can be predicted by their in vitro PBMC response to dexamethasone. This algorithm may further guide optimal medical treatment and possibly avoid the deleterious adverse effects of administering glucocorticoids to those patients who would not benefit from their effect.

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POTENTIALLY REVERSIBLE IDIOPATHIC sensorineural hearing loss (SNHL) affects approximately 5 to 20 of 100 000 patients annually[1]. The exact mechanisms leading to this disorder remain poorly understood. The 2 clinical syndromes, autoimmune inner ear disease (AIED), which is usually bilateral with a relapsing-remitting pattern, and sudden SNHL, which is typically an isolated unilateral event, are thought to be immune mediated. Inciting antigens in AIED are presumably self-antigens, while those of sudden SNHL are linked to a viral illness. Irrespective of the source of antigen, AIED and sudden SNHL likely converge in a similar inflammatory cascade leading to hearing loss.

The inflammatory cytokine milieu involved in immune-mediated SNHL (IM-SNHL) has not been extensively investigated. The molecular mechanisms behind the clinically observed effect of response and regain of hearing with glucocorticoid treatment are also unknown. Vari-

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ous cytokines have been implicated in IM-SNHL, such as interleukin (IL)-1β,[2] interferon (IFN)-γ,[3] and tumor necrosis factor (TNF; formerly known as tumor necrosis factor α).[4] Several clinical trials have been conducted in this patient population with immune-modulating agents such as methotrexate,[5] etanercept,[4,6,7] and infliximab,[8] all with limited success.

Ren et al[9] reported on TNF in patients with sudden SNHL and progressive SNHL in 1998. In a small study population of 15 subjects, they found that both TNF and IL-6 levels were significantly elevated in patients with sudden SNHL and progressive SNHL compared with control subjects. This was followed by a preliminary report by Rahman et al[7] on administering etanercept, a well-known TNF blocker used in autoimmune diseases such as rheumatoid arthritis and psoriasis, for patients with immune-mediated cochleovestibular disorders who did not respond to conventional therapies. They reported a 92% success rate (11 of 12 patients) in just hearing loss improvement with etanercept therapy. During the last decade, animal models of labyrinthitis have been used to investigate the role of TNF in recruiting inflammatory cells to the cochlea and its role in resultant hearing loss.[10] While some investigators reported that, in an animal model, etanercept has a protective effect on hearing loss,[11] others found it less effective than prednisone, with a greater potential for adverse effects.[4,6,12]

In 2 small human studies, levels of TNF in patients with sudden SNHL were found to be elevated in one study of 12 patients,[13] while the other study showed that both corticosteroid-responding and nonresponding patients (total of 30 for both groups together) had lower levels of TNF than controls and concluded that this cytokine does not offer useful information in either prognosis or diagnosis of the disease.[14] Likewise, the benefit of etanercept has not been consistently demonstrated. Pilot studies conducted by Matteson et al[6] in 23 patients showed that only 30% improved with etanercept therapy; however, 57% experienced no improvement and 13% worsened. In a study of similar size (20 patients), Cohen et al[4] failed to demonstrate any benefit of etanercept compared with placebo in maintaining hearing following benefit from corticosteroids. In 2006, Van Wijk et al[8] suggested from a study of 9 patients that locally administered etanercept into the middle ear allowed patients to be tapered off corticosteroid therapy and preserve their hearing function. None of these trials examined or used TNF level as a criterion for study enrollment.

To date, this is the largest study that characterizes TNF level in the peripheral circulation of patients with IM-SNHL. The effect of in vitro administration of dexamethasone on secreted TNF in peripheral blood mononuclear cells (PBMCs) to distinguish corticosteroid responsive patients is also investigated.

METHODS

HUMAN SUBJECTS

These studies were approved by the institutional review board at the North Shore–Long Island Jewish Health System and at the New York Eye and Ear Infirmary, and after obtaining informed consent, patients with AIED were recruited by 6 neurotologists (E.G., R.H., S.S.C., N.S., G.A., and A.V.) and had their blood drawn for PBMC isolation. Inclusion criteria were described previously[15,16] and were based on the AIED serial audiometry trial inclusion criteria.[17] Patients had SNHL of greater than 30 dB at 1 or more frequencies, with evidence of active decline in at least 1 ear of 15 dB at 1 frequency on their audiogram or 10 dB at 2 or more frequencies developing in more than 3 days but less than 90 days.[17] If the hearing loss evolved in less than 3 days, the patient displayed features suggestive of an autoimmune disorder. All patients were demonstrated to have no evidence of retrocochlear pathologic conditions, as determined by magnetic resonance imaging. Clinical response to oral glucocorticoids was defined as a 5-dB or greater average improvement at 250, 500, 1000, 2000, and 4000 Hz.[16] Patients with vestibular disorders in absence of hearing loss were excluded, even if they had systemic autoimmune disease. Clinical demographics of all the patients included in this study are listed in **Table 1**. Patients were categorized as steroid responders (n=47) and steroid nonresponders (n=38). Eleven healthy controls without any known autoimmune disease or history of hearing loss were included in the study for comparison with patients. The characteristics of the cohort are outlined in **Table 2**.

SAMPLE COLLECTION AND ISOLATION

Peripheral blood mononuclear cells were obtained from patients prior to clinical corticosteroid treatment and were isolated as previously described by density centrifugation of heparinized blood (Ficoll-Paque Plus; GE Healthcare Bio-Sciences AB).[15] The upper layer of plasma was collected and stored at −20°C until immediately prior to analysis.

HUMAN PBMC CULTURE AND STIMULATION

Isolated PBMCs were washed twice with 1 × RPMI (Gibco) and incubated in RPMI 1640 supplemented with 10% (volume/volume) fetal bovine serum (Atlanta Biologicals; a single lot of fetal bovine serum was used for all experiments), 100-U/mL penicillin G sodium salt, 100-µg/mL streptomycin sulfate, and 4.1mM glutamine, and plated at 1 × 106 cells/mL in 24-well plate (Costar). A portion of the PBMCs was incubated with 4-µg/mL dexamethasone sodium phosphate (APP Pharmaceuticals), and the remainder was left untreated for 16 hours at 37°C in 5% carbon dioxide. Optimal concentration of in vitro dexamethasone that maximized dose without compromising cell viability was previously determined.[15] Cell viability was measured after 16 hours and exceeded 80% in all cases. At the end of the incubation period, samples were centrifuged, and supernatants were collected and stored at −20°C. The pellets containing PBMCs were stored at −80°C until ready for use.

ELISA OF PLASMA SAMPLES AND CONDITION SUPERNATANTS

Plasma and supernatant TNF levels were quantified using a sandwich enzyme-linked immunosorbent assay (ELISA) (GE Healthcare, formerly Amersham Biosciences) as per the manufacturer's instructions. Plasma and condition supernatant samples were conducted on separate plates. The sensitivity of the assay was 2.5 pg/mL. An 8-point standard curve was constructed for each assay using a quadratic fit, and data were interpolated using BioLinx 2.2 software (Synatech Laboratories Inc). All samples were run in duplicate, and the mean variance was 0.023%.

Table 1. Clinical Demographic of Controls and Patients With Immune-Mediated Sensorineural Hearing Loss

Population Group/Patient No.	Sex/Age, y	Presence of Systemic Autoimmune Disease?
Control (n = 11), M:F, 3:8		
1C	M/51	No
2C	M/70	No
3C	F/58	No
4C	F/31	No
5C	F/55	No
6C	F/32	No
7C	F/45	Celiac disease
8C	F/31	No
9C	F/45	No
10C	M/36	No
11C	F/31	No
Responders (n = 47), M:F, 18:29		
1R	F/32	Hashimoto thyroiditis
2R	F/31	Multiple sclerosis
3R	F/57	No
4R	F/60	No
5R	M/14	No
6R	M/73	No
7R	M/62	CI
8R	F/60	CI
9R	F/25	No
10R	F/71	No
11R	F/38	No
12R	M/58	No
13R	M/86	No
14R	F/59	No
15R	F/56	No
16R	F/44	No
17R	F/33	CI; converted to nonresponder later; uveitis
18R	M/58	No
19R	F/14	No
20R	F/66	Psoriasis; monoclonal gammopathy
21R	F/50	No
22R	F/80	Crohn disease
23R	F/66	No
24R	F/43	No
25R	F/79	No
26R	F/37	No
27R	M/36	No
28R	F/58	Crohn disease
29R	M/4	CI
30R	M/61	No
31R	M/63	No
32R	F/66	Etanercept therapy
33R	F/43	No
34R	M/69	Neurodermatitis
35R	M/62	Psoriasis
36R	F/70	No
37R	M/66	No
38R	F/51	No
39R	F/52	No
40R	M/58	Sarcoidosis
41R	F/54	No
42R	M/62	No
43R	M/52	No
44R	F/59	No
45R	M/78	No
46R	F/22	CI
47R	M/47	No
Nonresponders (n = 38), M:F, 17:21		
1NR	M/58	No
2NR	F/51	No
3NR	F/63	CI
4NR	F/51	No
5NR	M/73	No
6NR	F/39	No
7NR	F/13	Muckle-Wells syndrome
8NR	F/55	No
9NR	F/60	Family history of Addison disease
10NR	F/75	CI
11NR	M/45	No
12NR	F/51	No
13NR	M/49	No
14NR	F/47	PCOS
15NR	M/45	CI
16NR	M/31	No
17NR	F/52	No
18NR	M/65	No
19NR	F/45	Multiple sclerosis
20NR	F/58	CI
21NR	M/56	CI
22NR	F/53	CI
23NR	F/66	No
24NR	M/40	No
25NR	M/61	No
26NR	M/73	No
27NR	F/54	No
28NR	F/56	Psoriasis; psoriatic arthritis
29NR	F/50	No
30NR	F/62	No
31NR	F/43	No
32NR	M/52	No
33NR	M/52	No
34NR	M/16	CI, previous responder
35NR	F/49	No
36NR	M/64	No
37NR	M/55	CI; type 1 diabetes mellitus
38NR	M/50	No

Abbreviations: CI, cochlear implant; F, female; M, male; PCOS, polycystic ovarian syndrome.

Table 2. Summary of Patient Characteristics

Population Group	Age Range (Mean), y	Male to Female Ratio
Control (n = 11)	31-70 (44.1)	1:2.7
Responders (n = 47)	4-86 (52.8)	1:1.6
Nonresponders (n = 38)	13-75 (52.1)	1:1.2

QUANTITATIVE REAL-TIME PCR OF DEXAMETHASONE-TREATED AND UNTREATED PBMCs

Quantitative real-time polymerase chain reaction (PCR) was performed using the ABI 7900HT Fast Real-time PCR System (Applied Biosystems); primer sequences, nucleotide position number, and gene bank accession numbers for actin and TNF gene were as described previously.[15,17] Quantitative real-time PCR conditions were as follows: 30 minutes at 48°C (1 cycle), 10 minutes at 95°C (1 cycle), 15 seconds at 95°C (45 cycles), and 1 minute at 60°C (45 cycles). For all PCRs, PCR mix contained 1 × master mix and 0.125 µL of Euroscript (Eurogentec RT qPCR MasterMix; Eurogentec) + reverse transcriptase inhibitor (0.125 U/µL) and ribonuclease inhibitor (0.05 U/µL). Polymerase chain reaction was then performed using the forward and reverse primers at a final concentration of 0.5µM in a sample volume of 25 µL. Relative quantification of the PCR signals was performed by comparing the cycle threshold value, in duplicate, of the gene of interest of each sample with the cycle threshold values of the reference gene actin. Quantitative real-time PCR analysis for each sample was performed in duplicate.

STATISTICAL ANALYSIS

Data groups were analyzed either by the Mann-Whitney test or by 1-way analysis of variance (ANOVA). Post hoc testing was performed using a Newman-Keuls multiple comparisons test. Two-way ANOVA was used for treatment vs patient group effect. $P \leq .05$ was considered statistically significant. Graph Pad Prism v5 (GraphPad Software Inc) was used for statistical analysis.

Table 3. Elevation of Plasma Tumor Necrosis Factor (TNF) Levels in Patients With Immune-Mediated Sensorineural Hearing Loss

	Plasma TNF Level, pg/mL	
Population Group	Mean (SEM)[a]	95% CI
Control (n = 10)	14.4 (1.7)[b]	10.6-18.3
Responders (n = 43)	24.1 (3.7)[c]	16.7-31.6
Nonresponders (n = 36)	27.6 (3.7)	20.1-35.0

[a] One-way analysis of variance, $P = .25$.
[b] Mann-Whitney test, $P = .03$ for controls vs (responders + nonresponders).
[c] Mann-Whitney test, $P = .08$ for responders vs nonresponders.

RESULTS

PLASMA TNF

Plasma from the control group subjects, clinical steroid responders and steroid nonresponders was analyzed for levels of TNF by ELISA. The mean (SEM) values for the controls, responders, and nonresponders were 14.4 (1.7), 24.1 (3.7), and 27.6 (3.7) pg/mL, respectively (**Table 3**). The range (95% CI) of plasma TNF values for each group was 9.7 to 27.7 pg/mL (10.6-18.3 pg/mL) for controls, 8.8 to 127.9 pg/mL (16.7-31.6 pg/mL) for responders and 10.5 to 105.2 pg/mL (20.1-35.0 pg/mL) for nonresponders. Analyzing for difference in plasma TNF levels between controls and patients (responders and nonresponders together) using the Mann-Whitney test showed a statistically significant difference between control and patient populations ($P = .03$); however, the difference between responders and nonresponders was nonsignificant ($P = .08$). Thus, measurement of plasma TNF in the presence of an acute decline in hearing is suggestive of IM-SNHL but is insufficient to predict response to corticosteroids.

PBMC-SECRETED TNF

Peripheral blood mononuclear cells from controls, responders, and nonresponders prior to clinical corticosteroid therapy were cultured in the presence of dexamethasone and compared with untreated cultures. The culture supernatants were analyzed for secreted TNF. Mean untreated (baseline) levels (SEM) of PBMC-secreted TNF were 10.0 (3.2), 36.1 (14.3) and 13.4 (2.0) pg/mL for controls, responders, and nonresponders, respectively (**Table 4**). The differences in these basal values were not found to be significant using 1-way ANOVA with Newman-Keuls multiple comparison tests and the Mann-Whitney test. The 95% confidence interval for the responder group was particularly wide (6.8-65.5 pg/mL), with a range of values from 0.0 to 372.0 pg/mL of TNF.

DEXAMETHASONE-TREATED PBMCs

Peripheral blood mononuclear cells from controls, responders, and nonresponders were isolated and were

Table 4. Elevation of Tumor Necrosis Factor (TNF) Level in the Supernatants of Steroid-Responsive Immune-Mediated Sensorineural Hearing Loss

	Plasma TNF Level, pg/mL			
Population Group	Untreated, Mean (SEM)[a]	95% CI	Dexamethasone Treated, Mean (SEM)[d]	Mean Fold Change (SEM)[e]
Control (n = 9)	10.0 (3.2)[b]	2.5-17.5	6.8 (2.4)	0.73 (0.30)
Responders (n = 27)	36.1 (14.3)[c]	6.8-65.5	8.2 (1.4)	1.05 (0.37)
Nonresponders (n = 26)	13.4 (2.0)	9.1-17.7	12.6 (2.1)	1.15 (0.22)

[a] One-way analysis of variance (ANOVA), $P = .20$.
[b] Mann-Whitney test $P = .28$ for controls vs (responders +nonresponders).
[c] Mann-Whitney test, $P = .80$ for responders vs nonresponders.
[d] Two-way ANOVA, for treatment $P = .15$.
[e] One-way ANOVA, $P = .79$.

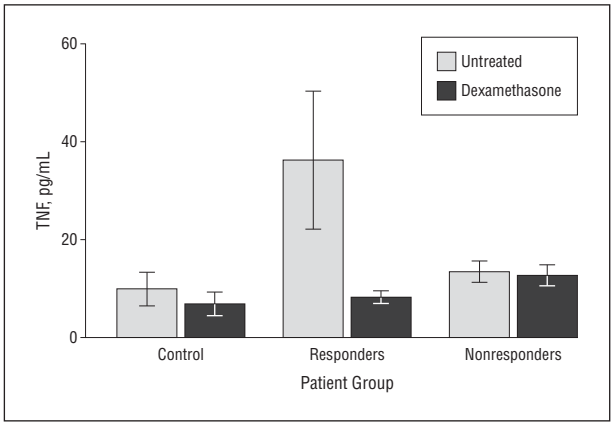

Figure 1. Peripheral blood mononuclear cell (PBMC)-secreted tumor necrosis factor (TNF): in vitro dexamethasone treatment effect. In all 3 groups, dexamethasone use decreased TNF level. The differences among patient groups (P = .27) and among treated and untreated PBMCs (P = .15) are not statistically significant. Error bars designate SEMs.

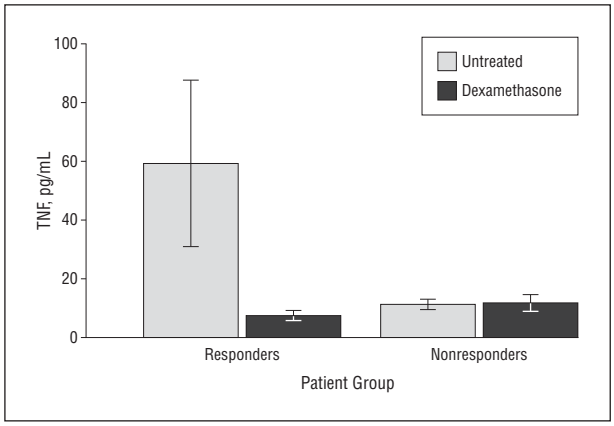

Figure 2. Patients with high plasma levels of tumor necrosis factor (TNF): effect of in vitro dexamethasone on peripheral blood mononuclear cells (PBMCs). In vitro response of PBMCs to dexamethasone is reflected by the clinical response to oral glucocorticoids in those patients who have a high plasma levels of TNF (>14.4 pg/mL). The difference among responders and nonresponders is statistically significant (P = .04). Error bars designate SEMs.

Table 5. Patients With High Plasma[a] Tumor Necrosis Factor (TNF) Level[b]

Population Group[c]	Plasma TNF Level, Mean (SEM), pg/mL	
	Untreated	Dexamethasone Treated
Responders (n = 13)	59.1 (28.5)	7.2 (2.1)
Nonresponders (n = 18)	11.2 (1.6)	11.7 (2.7)

[a] Defined as baseline plasma TNF level greater than the control group mean baseline plasma TNF level (14.4 pg/mL).
[b] In vitro dexamethasone-treated peripheral blood mononuclear cells parallel the expected clinical response.
[c] Two-way analysis of variance for population group, P = .04.

either left untreated or treated with dexamethasone overnight. The resulting supernatants were analyzed for secreted TNF. Dexamethasone treatment of PBMCs resulted in reduced TNF secretion in all groups, as demonstrated by a mean (SEM) secretion of 10.0 (3.2) pg/mL in untreated PBMCs to 6.8 (2.4) pg/mL in dexamethasone-treated PBMCs of controls, 36.1 (14.3) pg/mL to 8.2 (1.4) pg/mL in clinical steroid responders, and 13.4 (2.0) pg/mL to 12.6 (2.1) pg/mL, for nonresponders, respectively (Table 4 and **Figure 1**). The differences in these values were not found to be statistically significant using 2-way ANOVA for either the population group (P = .27) or treatment (P = .15). The mean (SEM) fold change from baseline, untreated PBMC-secreted TNF was 0.73 (0.30) for the control group, 1.05 (0.37) for the responders, and 1.15 (022) for the nonresponders. These differences were not found to be statistically significant (P = .79 with 1-way ANOVA).

PBMCs TREATED WITH DEXAMETHASONE IN PATIENTS WITH HIGH PLASMA TNF LEVELS

We separately analyzed TNF secretion in the cell culture supernatant from PBMC from patients whose plasma TNF levels were elevated (greater than the mean of the control group [>14.4 pg/mL]). There were 13 available dexamethasone–treated PBMC culture supernatants from patients from the responder group and 18 from the nonresponder group. The mean (SEM) TNF level in PBMC culture supernatants from the high plasma TNF clinical corticosteroid-responder group, was 59.1 (28.5) pg/mL in untreated PBMC supernatant, which decreased to 7.2 (2.1) pg/mL with the addition of dexamethasone. The mean (SEM) TNF level in the high plasma TNF nonresponder group untreated PBMC supernatant was 11.2 (1.6) pg/mL, which slightly increased to 11.7 (2.7) pg/mL with the addition of dexamethasone (**Table 5** and **Figure 2**). The difference with respect to patient population was statistically significant (P = .04 with 2-way ANOVA analysis).

TNF mRNA IN PBMCs

Peripheral blood mononuclear cells were isolated from select controls (n = 4), responders (n = 7), and nonresponders (n = 10). They were subsequently left untreated or were treated in vitro with dexamethasone, RNA isolated, and TNF transcription analyzed. The mean (SEM) total number of quantitative real-time PCR cycles (inversely proportional to the amount of mRNA present) was 29.5 (1.2), 28.4 (0.3), and 29.2 (0.2) for the control, responder, and nonresponder patient groups, respectively (data not shown). This difference was not statistically significant (P = .34, 1-way ANOVA). Glucocorticoid use decreased the amount of TNF transcribed by 0.59 (0.12)–fold, 0.49 (0.11)–fold, and 0.53 (0.10)–fold from the untreated PBMCs in control, responder, and nonresponder groups, respectively (data not shown). This glucocorticoid effect was not statistically significant for the small difference observed among the groups (P = .85). There is an obviously a discrepancy in the observed dexamethasone effect on the TNF mRNA transcription and on the TNF protein secretion, which can only be explained by a downstream event, such as posttranslational modification.

COMMENT

The cytokine environment that governs steroid responsiveness in patients with IM-SNHL is yet to be elucidated. Very few studies, mostly limited by small sample size, have investigated the role of TNF and its inhibitors in IM-SNHL, which is thus far inconclusive. Our study has the advantage of a large sample size and potentially important implications in both diagnostic and prognostic value of TNF in steroid-responsive IM-SNHL.

The following conclusions can be drawn from the presented data:

1. In a patient presenting with hearing loss, plasma TNF levels higher than 18.3 pg/mL may establish a diagnosis of IM-SNHL with 97% certainty (the upper boundary of the 95% confidence interval for control subjects; **Table 6**). The higher threshold for diagnosing the disease, however, comes at a cost of very low sensitivity (46.8%). Therefore, plasma TNF cannot be used as a screening test, but rather a confirmatory test in those patients who are suspected to have IM-SNHL based on their clinical presentation. Even with lowering the threshold to greater than 14.4 pg/mL (the mean value of control subjects), the sensitivity of the test is still too low for use in screening (data not shown). In a patient with suspected IM-SNHL, a peripheral venous plasma level of TNF alone cannot predict their response to corticosteroids in the ability to recover hearing, however.

Table 6. Specificity and Sensitivity of Plasma Tumor Necrosis Factor (TNF) Level as a Diagnostic Marker for Immune-Mediated Sensorineural Hearing Loss[a]

TNF >18.3 pg/mL	Condition Positive	Condition Negative	PPV/NPV
Positive result	True positive, 18 + 19 = 37	False positive, 1	**PPV,** 37/(37 + 1) = **97.4%**
Negative result	False negative, 25 + 17 = 42	True negative, 9	**NPV,** 9/(9 + 42) = **17.6%**
	Sensitivity, 37/(37 + 42) = **46.8%**	**Specificity,** 9/(9 + 1) = **90.0%**	

Abbreviations: NPV, negative predictive value; PPV, positive predictive power.
[a] Threshold set at upper limit of the 95% confidence interval of control subjects.

2. By stratifying those patients whose plasma level of TNF is high (defined as higher than the mean of the control group [14.4 pg/mL]) the clinician could predict a patient's clinical response to corticosteroids by stimulating PBMCs with dexamethasone. If there is a reduction in TNF secretion with in vitro dexamethasone, then the patient will likely clinically respond to oral corticosteroid therapy; if there is no reduction with in vitro dexamethasone, then the oral corticosteroid therapy will likely not be of benefit (**Figure 3**). This is an important finding, since this algorithm could be used to spare patients the potential and even serious adverse effects of high-dose oral glucocorticoid therapy.

3. While the TNF mRNA transcription is reduced with the addition of dexamethasone to PBMCs in all 3 control groups, this effect is not seen by ELISA on circulating TNF in the plasma or on secreted by PBMCs. Likely, there is a posttranscriptional alteration of TNF expression that is not affected by glucocorticoids.

4. The presented data may offer an explanation for the weak clinical effect with etanercept for hearing restoration in this disease. In both the clinical studies by Matteson et al[6] and Cohen et al,[4] prior to administering etanercept, the patients were treated with oral corticosteroids. Oral corticosteroids can certainly reduce the levels of TNF in corticosteroid responders, as seen by our data. Once TNF levels are already suppressed, etanercept may no longer be able to exert a significant clinical effect on an already suppressed cytokine. Alternatively, measurement of TNF plasma and/or culture supernatant levels and treatment of only those "high expressers" may afford improved audiometric responsiveness to TNF antagonist therapies. Clearly, clinical trials correlating TNF level with clinical response would be necessary to answer this question.

While our study has the benefit of a larger group of patients, TNF level remains highly variable in patients with IM-SNHL, emphasizing that clinical IM-SNHL likely represents a spectrum of disorders mediated by different cytokines. Interestingly, TNF levels can be modulated with corticosteroids, highlighting the role of TNF in the corticosteroid-responsive subset of patients. In patients with a clinical diagnosis of IM-SNHL, although 70% are initially corticosteroid responsive, that response is lost over time: only 14% are responsive after 34 months.[18] This transition to a corticosteroid-resistant phenotype is likely a result in a change in the local cytokine microenviron-

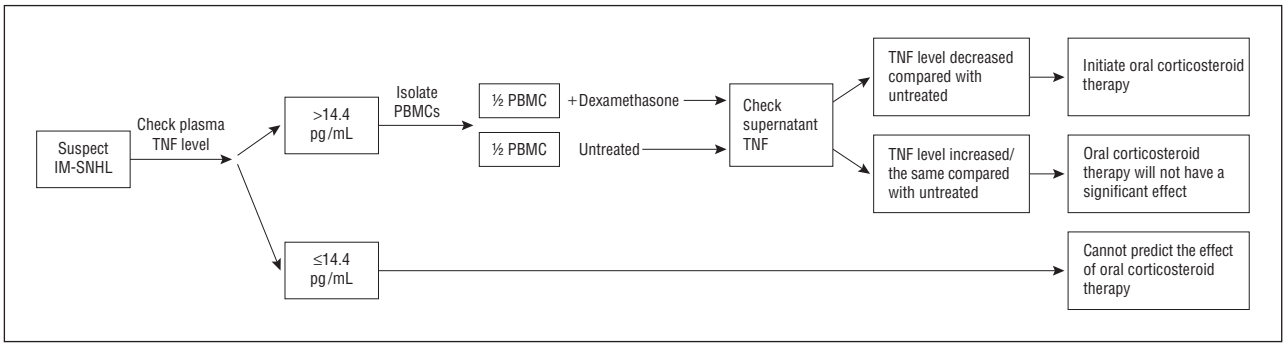

Figure 3. Prognostic algorithm of oral corticosteroid therapy based on plasma levels of tumor necrosis factor (TNF). IM-SNHL indicates immune-mediated sensorineural hearing loss; and PBMCs, peripheral blood mononuclear cells.

ment. In support of this hypothesis, our laboratory has demonstrated the critical role and overexpression of IL-1β in corticosteroid-resistant IM-SNHL.[15,16] This overexpression of IL-1β has formed the basis of our phase 1 clinical trial of anakinra in corticosteroid-resistant autoimmune hearing loss (NCT01267994). Our results are suggestive that TNF has a more substantial predictive role in those patients with IM-SNHL who are corticosteroid responsive and consistent with our prior results showing that IL-1β appears to dictate the events in steroid-resistant hearing loss.

In conclusion, TNF has the potential of being used as both a diagnostic marker for IM-SNHL and prognostic biomarker for corticosteroid response in this disease.

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Conflict of Interest Disclosures: Dr Chandrasekhar is a shareholder and board member of Scientific Development and Research Inc, a small biotech company involved in surfactant research. The Feinstein Institute for Medical Research is in the process of filing a patent for the measurement of TNF as a measure of steroid responsiveness in immune-mediated sensorineural hearing loss.
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