SCREENING FOR TUBERCULOSIS IN AN OUTPATIENT POPULATION OF MULTIPLE SCLEROSIS

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ABSTRACT

BACKGROUND: Up to 13 million people have latent tuberculosis infection (LTBI) in the USA. Patients with a compromised immune system are at risk for converting from LTBI to active tuberculosis infection. Immunomodulatory therapies for multiple sclerosis (MS) may put individuals with LTBI at higher risk of developing tuberculosis.

OBJECTIVES: We aimed to screen patients with MS for LTBI to stratify their risk of developing tuberculosis on immunosuppressive agents. We then used laboratory data to determine if immunosuppressive agents compromise the validity of tuberculosis screening.

METHODS: Patients of the Beth Israel Deaconess Medical Center's Multiple Sclerosis Center in Boston, MA were screened for tuberculosis as part of routine testing with the QuantiFERON-TB Gold In-Tube (QFT-GIT) assay (Cellestis Limited) from December 2013 to December 2017. Patients were tested either prior to initiating therapy or while on immunomodulating therapy. Data were analyzed using JMP Pro 13.0.0 (SAS, Cary, NC). Wilcoxon rank sum was used to compare non-parametric continuous data. Pearson's $\chi 2$ was used to compare categorical data; Fisher's exact test was used when any cell contained fewer than 10 observations.

RESULTS: 4 out of 225 patients (1.8%) had positive QFT-GIT testing; 2 of these patients had no known risk factors for tuberculosis, one patient had emigrated from Russia, and the last patient had emigrated from China and had been treated for tuberculosis in the past. 30 out of 225 patients (13.3%) had an indeterminate assay result. 21 of the 30 indeterminate results (70.0%) occurred in patients taking dimethyl fumarate (DMF). Indeterminate assay results were significantly more likely to be associated with DMF use, lymphocytopenia (driven by grade 2 lymphocytopenia) , decreased absolute lymphocyte count, decreased CD3 count, decreased CD4 count, decreased CD8 count, and increased CD4 to CD8 ratio.

CONCLUSIONS: LTBI may be more common than expected, even without known risk factors for tuberculosis. Screening for LTBI prior to starting immunosuppressive agents for MS could help prevent activation of tuberculosis. DMF lowers the reliability of the QFT-GIT assay, presumably due to a functional lymphocytopenia and alteration in cytokines, such as interferon gamma. In contrast, patients on fingolimod had a high rate of lymphocytopenia without compromising the GFT-GIT assay in most cases.

BACKGROUND

- 13 million people have latent tuberculosis infection (LTBI) in the USA.
- 5-10% of individuals with LTBI will progress to infectious tuberculosis (TB).
- Treating LTBI reduces the chance of developing active TB by 90%.
- Patients with a compromised immune system are at a higher risk for converting from LTBI to active TB infection.
- The QuantiFERON-TB Gold In-Tube (QFT-GIT) assay is an interferon-gamma release assay (IGRA) that measures the T-cell release of interferon-gamma (IFN-γ) in response to *Mycobacterium tuberculosis* antigens.
- Dimethyl fumarate (DMF) is an MS immunomodulatory therapy that has anti-inflammatory and neuroprotective effects mediated by:
  - Activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) transcriptional pathway.
  - Decreased migratory activity of immune cells.
  - Reduction in lymphocyte count and cytokine production (IFN-γ, granulocyte-macrophage colony-stimulating factor, TNF-α, IL-17, etc.).

RESULTS

Table 1. Tuberculosis Test Results

Positive	4/225 (1.8)[a]
Negative	191/225 (84.9)
Indeterminate	30/225 (13.3)

[a] Two of these patients had no known risk factors for tuberculosis, one patient had emigrated from Russia, and the last patient had emigrated from China and had been treated for tuberculosis in the past

Table 2. Characterization of Tuberculosis Results

	Indeterminate Result		Diagnostic Result			
	n/N	(%)	n/N	(%)	P Value	OR (95% CI)
Female Sex	16/30	(53.3)	131/195	(67.2)	0.138	0.56 (0.26-1.2)
Age, median (range)	49	(30, 74)	47	(18, 82)	0.079	-
Current Immunomodulating Therapy	25/30	(83.3)	113/195	(58.0)	**0.008**	3.6 (1.3-9.9)
History of Immunomodulating Therapy	27/30	(90.0)	138/195	(70.8)	**0.027**	3.7 (1.1-12.7)
Current Dimethyl Fumarate	21/30	(70.0)	39/195	(20.0)	**<0.001**	9.3 (4.0-22.0)
History of Dimethyl Fumarate	24/30	(80.0)	56/195	(28.7)	**<0.001**	9.9 (3.9-25.6)
Current Fingolimod	1/30	(3.3)	12/195	(6.2)	1.000	0.53 (0.07-4.2)
Current Treatment Duration, years (IQR)	2.1	(1.1, 3.0)	2.4	(1.2, 3.7)	0.111	-
White Blood Cell Count, K/µL, median (IQR)	5.3	(4.6, 9.6)	6.4	(5.0, 8.1)	0.487	-
% Lymphocytes, median (IQR)	12.3	(8.4, 18.1)	26.7	(21.2, 32.8)	**<0.001**	-
Absolute Lymphocyte Count per µL, median (IQR)	733	(568, 1066)	1764	(1210, 2280)	**<0.001**	-
CD3 per µL, median (IQR)	462	(285, 634)	1269	(852, 1702)	**<0.001**	-
CD4 per µL, median (IQR)	360	(227, 508)	860	(597, 1179)	**<0.001**	-
CD8 per µL, median (IQR)	85	(54, 168)	356	(182, 540)	**<0.001**	-
CD4/CD8 Ratio	3.64	(2.28, 5.65)	2.58	(1.59, 3.53)	**0.002**	-
Lymphocytopenia	21/30	(70.0)	29/192	(15.1)	**<0.001**	13.1 (5.5-31.5)
Lymphocytopenia (Grade 1)	3/30	(10.0)	10/192	(5.2)	0.392	2.0 (0.52-7.8)
Lymphocytopenia (Grade 2)	15/30	(50.0)	12/192	(6.3)	**<0.001**	15.0 (5.6-37.8)
Lymphocytopenia (Grade 3)	3/30	(10.0)	6/192	(3.1)	0.107	3.4 (0.81-14.6)
Lymphocytopenia (Grade 4)	0/30	(0.0)	1/192	(0.5)	1.000	N/A

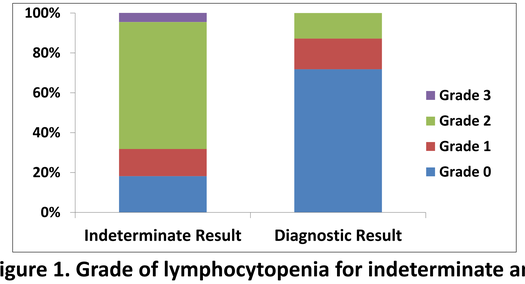

Figure 1. Grade of lymphocytopenia for indeterminate and diagnostic results for patients on dimethyl fumarate

Table 3. Characterization of Tuberculosis Results for Patients on Dimethyl Fumarate

	Indeterminate Result		Diagnostic Result			
	n/N	(%)	n/N	(%)	P Value	OR (95% CI)
Female Sex	12/21	(57.1)	30/39	(76.9)	0.144	0.40 (0.13-1.3)
Age, median (range)	52	(38, 63)	49	(18, 70)	0.223	-
Current Treatment Duration, years (IQR)	2.4	(1.4, 3.1)	2.3	(1.5, 3.2)	0.883	-
White Blood Cell Count, K/µL , median (IQR)	5.1	(4.4, 6.8)	5.1	(4.4, 6.6)	0.727	-
% Lymphocytes, median (IQR)	13.3	(10.1, 18.5)	23.3	(17.6, 28.4)	**<0.001**	-
Absolute Lymphocyte Count per µL, median (IQR)	714	(587, 925)	1239	(984, 1674)	**<0.001**	-
CD3 per µL, median (IQR)	390	(266, 575)	826	(672.5, 1220.3)	**<0.001**	-
CD4 per µL, median (IQR)	351	(226.5, 467.5)	623	(504.3, 858.3)	**<0.001**	-
CD8 per µL, median (IQR)	68	(38.5, 107)	208	(98.8, 349)	**<0.001**	-
CD4/CD8 Ratio	4.63	(2.82, 6.88)	2.89	(2.23, 4.85)	**0.035**	-
Lymphocytopenia	17/21	(81.0)	11/39	(28.2)	**<0.001**	10.8 (3.0-39.4)
Lymphocytopenia (Grade 1)	2/21	(9.5)	6/39	(15.4)	0.701	0.58 (0.11-3.2)
Lymphocytopenia (Grade 2)	14/21	(66.7)	5/39	(12.8)	**<0.001**	13.6 (3.7-50.2)
Lymphocytopenia (Grade 3)	1/21	(4.8)	0/39	(0.0)	0.350	N/A
Lymphocytopenia (Grade 4)	0/21	(0.0)	0/39	(0.0)	-	N/A

Table 4. Breakdown of Indeterminate Results and Lymphocytopenia by Current Therapy

	Indeterminate Results, n/N (%)	Lymphocytopenia, n/N (%)	Lymphocytopenia for Indeterminate Results, n/N (%)
No therapy	5/87 (5.8)[a]	3/86 (3.5)	1/5 (20.0)
Dimethyl fumarate	21/60 (35.0)[b]	28/60 (46.7)	17/21 (81.0)
Fingolimod	1/13 (7.7)	12/13 (92.3)	1/1 (100.0)
Injectables	1/37 (2.7%)[c]	4/35 (11.4)	1/1 (100.0)
Methotrexate	1/1 (100.0)	1/1 (100.0)	1/1 (100.0)
Methylprednisolone	1/2 (50.0)[d]	0/2 (0.0)	0/1 (0.0)
Natalizumab	0/10 (0.0)	0/10 (0.0)	N/A
Anti-CD20 Therapy	0/10 (0.0)	1/10 (10.0)	N/A
Teriflunomide	0/5 (0.0)	1/5 (20.0)	N/A

[a] 3 of the 5 indeterminate results had repeat testing with negative results.
[b] 1 patient with an indeterminate result was retested 3 months after discontinuation of dimethyl fumarate, and the result was still indeterminate. This patient did not have lymphocytopenia during either test.
[c] This patient was being treated with pegginterferon beta-1a after being switched from dimethyl fumarate due to lymphocytopenia. The patient had persistent lymphocytopenia at the time of testing, which was 9 months after discontinuing the dimethyl fumarate. Repeat testing was negative while the patient was being treated with rituximab.
[d] Testing was performed on day 3 of 5 of intravenous methylprednisolone for an acute flare. Repeat testing was negative.

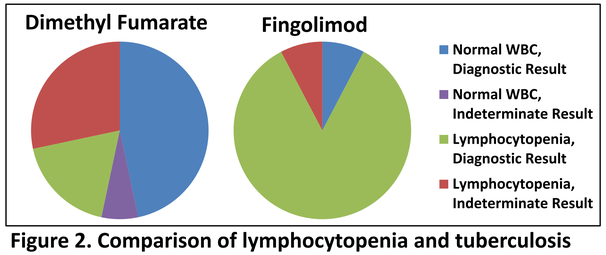

Figure 2. Comparison of lymphocytopenia and tuberculosis results for patients on dimethyl fumarate and fingolimod

METHODS

- Patients were screened for TB using the QFT-GIT assay (Cellestis Limited) from December 2013 to December 2017.
- Patients were tested either prior to initiating therapy or while on immunomodulating therapy.
- Data were analyzed using JMP Pro 13.0.0 (SAS, Cary, NC).
  - Wilcoxon rank sum was used to compare non-parametric continuous data.
  - Pearson's $\chi 2$ was used to compare categorical data.
  - Fisher's exact test was used when any cell contained fewer than 10 observations.
- Definitions:
  - "Diagnostic" results include positive and negative results.
  - "Injectables" include glatiramer acetate and interferon therapies.
  - "Anti-CD20 therapy" includes rituximab and ocrelizumab.
  - Lymphocytopenia grades were defined using the National Cancer Institute Common Toxicity Criteria [Grade 1: 800-999/mm^3; Grade 2: 500-799/mm^3; Grade 3: 200-499/mm^3; Grade 4: <200/mm^3].

CONCLUSIONS

- Routine screening in our MS population revealed 4 positive results for TB using QFT-GIT testing (1.8%). LTBI may be more common than expected.
- Screening for LTBI and treating appropriately prior to starting immunosuppressive agents for MS could help prevent activation of tuberculosis.
- Indeterminate results were associated with DMF use, lymphocytopenia (driven by grade 2 lymphocytopenia), decreased absolute lymphocyte count, decreased CD3 count, decreased CD4 count, decreased CD8 count, and increased CD4 to CD8 ratio.
- 35% of DMF patients had indeterminate results, presumably due to a functional lymphocytopenia and alteration in cytokines (IFN-γ).
- Fingolimod patients had a high rate of lymphocytopenia without compromising the QFT-GIT assay, stressing the different mechanism of actions.

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