

1 ***Demographics of controls with index sample antibody positivity***

2       The 50 controls with Tg antibody or TPO antibody positivity at the index sample  
3 are summarized demographically in Supplement Table 1. No controls were TSH-R  
4 antibody positive at the index or pre-3 samples.

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6 ***Validation of Index Date as the Diagnosis Date***

7       To validate the index date as the diagnosis date for Graves disease or Hashimoto  
8 thyroiditis, we collected any clinical procedural terminology (CPT) laboratory codes  
9 among outpatient encounters for detection of thyroid antibodies prior to the index date  
10 (CPT codes 84432, 84436, 84439, 84442, 84443, 84479, 84480). If the index date,  
11 identified by the first ICD-9-CM code for Graves disease or Hashimoto thyroiditis, is a  
12 good surrogate of the clinical diagnosis date, relatively few cases or controls should have  
13 CPT codes for thyroid antibody detection prior to the index date. Fourteen cases (7  
14 Graves disease cases and 7 Hashimoto thyroiditis) had CPT codes present before  
15 diagnosis. The first code was found a median of 282 days before diagnosis (range 4 –  
16 376). Of the controls, six had CPT codes for Graves disease or Hashimoto thyroiditis  
17 laboratory tests. The first code was recorded a median of 386 days before the index date  
18 (range 158 – 644). The clinical characteristics of these patients are described in  
19 Supplement Table 2. When these cases and controls were excluded, the results were  
20 consistent with those reported.

21       We also looked for ICD-9-CM codes in cases prior to the index date. No previous  
22 ICD-9-CM codes for Graves disease or Hashimoto thyroiditis were identified in  
23 Hashimoto thyroiditis cases or any controls. We did identify 3 Graves disease cases with

24 ICD-9-CM codes prior to the index date. Among these cases, the first code was observed  
25 a median of 30 days before the index date diagnosis (range 5 – 429). When we excluded  
26 these 3 cases and their controls, the results were consistent with those reported.

### 27 ***Sensitivity, specificity and predictive value of antibody positivity***

28 Sensitivity, specificity and predictive value of the thyroid antibodies individually  
29 and together were calculated at each time point with case status serving as the gold  
30 standard. To test how well a pre-diagnostic measurement could serve as a marker of  
31 future disease, we calculated the sensitivity and specificity of thyroid antibody positivity  
32 at all time points (Supplement Tables 3 and 4). We calculated sensitivity and specificity  
33 for antibody positivity to Tg, TPO or TSH-R separately; either Tg or TPO antibody  
34 positive; both Tg and TPO antibody positive, either Tg, TPO or TSH-R antibody  
35 positive; or Tg, TPO and TSH-R antibody positive. The sensitivity and specificity of Tg  
36 or TPO antibody positivity was similar up to 7 years prior to diagnosis as at diagnosis in  
37 Hashimoto thyroiditis (Sensitivity pre-3: 72, diagnosis: 74; Specificity pre-3: 86,  
38 diagnosis: 86), but not Graves disease (Sensitivity pre-3: 38, diagnosis: 64; Specificity  
39 pre-3: 86, diagnosis: 86). Combining antibody positivity for Tg, TPO or TSH-R at the  
40 diagnostic sample yielded a higher sensitivity but similar specificity than the combination  
41 of Tg or TPO antibody positivity (Sensitivity 75% vs 64%; Specificity 88% vs 86%).  
42 However, the sensitivity and specificity at the pre-3 time point was similar for Tg, TPO  
43 or TSH-R and Tg or TPO antibody positivity (Sensitivity 38 vs 38; Specificity 88 vs 86).

### 44 ***Change in antibody levels within person over time***

45 A repeated-measures linear model was created using PROC MIXED with  
46 unstructured variance to analyze the change in the levels of Tg and TPO antibody to

47 disease diagnosis date. This model accounts for the within-person variability that occurs  
48 when multiple measures are taken from the same person as well as the between-person  
49 variability.

50 *Graves disease cases versus controls.* Figure 1 in the manuscript demonstrates  
51 the increase in thyroid antibodies over time among Graves disease cases. Comparing the  
52 mean antibody levels in Graves disease cases to controls (Supplement Table 5),  
53 accounting for within person variability in Tg antibody measures over time, the pre-3  
54 serum levels are not statistically different between cases and controls. Yet the rate of  
55 change over time is significantly greater in cases than controls ( $p < 0.001$ ). When the same  
56 comparison is made for TPO antibodies, the pre-3 TPO antibodies are not statistically  
57 different between Graves disease cases and controls. The rate of change is significantly  
58 different ( $p < 0.0001$ ). The rate of change of TPO antibodies in Graves disease cases over  
59 time is 122 times greater than the rate of change in controls.

60 *Hashimoto thyroiditis cases versus controls.* For Tg and TPO antibodies, the  
61 Hashimoto thyroiditis pre-3 serum levels are significantly elevated in cases compared to  
62 controls as diagrammed in Manuscript Figure 2 and Supplement Table 5 ( $p < 0.0001$ ).  
63 The pre-3 Tg antibodies in patients were 259 units higher than in the controls. The pre-3  
64 TPO antibodies in cases were 590 units higher than controls ( $p < 0.0001$ ). The rate of Tg  
65 or TPO antibody change over time was not significantly different between cases and  
66 controls ( $p > 0.05$ ).

67

68 **Supplement Table 1. Description of the controls with Tg or TPO antibody positivity**  
 69 **at the index sample.\***

	<b>Tg+ only</b>	<b>TPO+ only</b>	<b>TPO+ &amp; Tg+</b>
<b>N</b>	9	21	20
<b>Age at index</b>	36	32	32
<i>Median</i>	(23 – 50)	(24 – 45)	(24 – 45)
<i>(Min – Max)</i>			
<b>Race-Ethnicity N</b>			
White	4	13	11
African American	3	5	3
Hispanic	1	2	4
Other	0	1	2
Clinical thyroid antibody testing prior to index date N	0	0	1**
<b>Tg antibody <i>Median</i></b> (Minimum - Maximum)			
Pre-3	<i>175.7</i>	<i>34.2</i>	<i>126.4</i>
	28.7 – 622.5	11.9 – 423.7	10.4 – 1111.7
Pre-2	<i>189.7</i>	<i>37.0</i>	<i>165.5</i>
	23.4 – 672.7	6.5 – 290.5	29.9 – 1078.3
Pre-1	<i>164.0</i>	<i>41.2</i>	<i>164.1</i>
	7.5 – 1414.3	9.0 – 198.0	31.5 – 1371.4
Index	<i>229.2</i>	<i>40.0</i>	<i>231.2</i>
	101.7 – 762.1	12.1 – 96.4	123.7 – 1084.3
<b>TPO antibody <i>Median</i></b> (Minimum - Maximum)			
Pre-3	<i>11.0</i>	<i>968.2</i>	<i>285.6</i>
	0 – 58.6	0 – 1876.5	0 – 1650.0
Pre-2	<i>0</i>	<i>640.1</i>	<i>558.2</i>
	0 – 102.6	0 – 1910.2	88.0 – 1398.4
Pre-1	<i>0</i>	<i>710.1</i>	<i>651.7</i>
	0 – 81.1	106.5 – 1904.8	28.0 – 1671.0
Index	<i>0</i>	<i>518.2</i>	<i>1081.1</i>
	0 – 15.7	100.3 – 1761.4	137.2 – 1629.7

70 \*No control was TSH-R antibody positive.

71 \*\*ID 3 in Supplement Table 2

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73 Tg, thyroglobulin; TPO, thyroperoxidase; TSH-R, thyroid stimulating hormone receptor

**Supplement Table 2. Description of the six controls with CPT codes for thyroid testing prior to the index date.\***

<b>ID</b>	<b>Test performed</b>	<b>Age at index</b>	<b>Days between first CPT code and index</b>	<b>Race-Ethnicity</b>	<b>Pre-3 Tg Abs</b>	<b>Index Tg Abs</b>	<b>Pre-3 TPO Abs</b>	<b>Index TPO Abs</b>
1	TSH	42	1,366	Unknown	18.0	14.3	4.1	0
2	TSH	30	386	White	40.2	42.5	0	0
3	TSH	28	1,106	White	10.4	124.9	0	137.2
4	TSH, Thyroxine	35	45	African American	13.9	14.3	0	0
5	TSH, Thyroxine	33	204	White	26.4	12.4	0	0
6	TSH	43	158	African American	17.2	14.2	0	0

\*None of these controls was selected for the random sample for TSH-R antibody testing.

Abs, antibodies; Tg, thyroglobulin; TPO, thyroperoxidase; TSH, thyroid stimulating hormone

**Supplement Table 3. Sensitivity and specificity of Tg, TPO and TSH-R antibody positivity at different time points for differentiating Graves disease from controls.**

	Thyroglobulin+				Thyroid Peroxidase+				TSH-R+				Tg+ OR TPO+				Tg+ AND TPO+				Tg+, TPO + or TSH-R+		Tg+, TPO+ and TSH-R+	
	Pre 3	Pre2	Pre 1	Dx	Pre 3	Pre 2	Pre 1	Dx	Pre 3	Pre 2	Pre 1	Dx	Pre 3	Pre 2	Pre 1	Dx	Pre 3	Pre 2	Pre 1	Dx	Pre 3	Dx	Pre 3	Dx
<b>Controls, % positive</b>	7	8	8	<b>8</b>	11	11	13	<b>12</b>	0			<b>0</b>	14	14	16	<b>14</b>	5	5	5	<b>6</b>	12	<b>12</b>	0	<b>0</b>
<b>Graves disease, % positive</b>	18	29	29	<b>47</b>	31	36	49	<b>57</b>	2	7	20	<b>55</b>	38	46	56	<b>64</b>	11	18	22	<b>40</b>	38	<b>75</b>	2	<b>25</b>
<b>Sens, %</b>	18	29	29	<b>47</b>	31	36	49	<b>57</b>	2			<b>55</b>	38	46	56	<b>64</b>	11	18	22	<b>40</b>	38	<b>75</b>	2	<b>25</b>
<b>Spec, %</b>	93	92	92	<b>92</b>	89	89	87	<b>88</b>	100			<b>100</b>	86	86	84	<b>86</b>	95	95	95	<b>94</b>	88	<b>88</b>	100	<b>100</b>
<b>PPV, %</b>	39	48	46	<b>59</b>	40	44	49	<b>55</b>	100			<b>100</b>	40	45	47	<b>53</b>	38	46	51	<b>64</b>	94	<b>97</b>	100	<b>100</b>
<b>NPV, %</b>	82	84	84	<b>87</b>	84	85	87	<b>89</b>	17			<b>30</b>	85	86	89	<b>91</b>	81	82	83	<b>86</b>	22	<b>41</b>	17	<b>20</b>

Tg, thyroglobulin antibody; TPO, thyroperoxidase antibody; TSH-R, thyroid stimulating hormone receptor antibody; Sens, sensitivity;

Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; Dx, diagnosis

**Supplement Table 4. Sensitivity and specificity of Tg and TPO antibody positivity at different time points for differentiating Hashimoto thyroiditis from controls.**

	Thyroglobulin+				Thyroid Peroxidase+				Tg+ OR TPO+				Tg+ AND TPO+			
	Pre3	Pre2	Pre1	Dx	Pre3	Pre2	Pre1	Dx	Pre3	Pre2	Pre1	Dx	Pre3	Pre2	Pre1	Dx
Controls, % positive	7	8	8	<b>8</b>	11	11	13	<b>12</b>	14	14	16	<b>14</b>	5	5	5	<b>6</b>
Hashimoto thyroiditis, % positive	53	52	51	<b>57</b>	67	66	68	<b>66</b>	72	71	75	<b>74</b>	47	46	44	<b>49</b>
Sens, %	53	52	51	<b>57</b>	67	66	68	<b>66</b>	72	71	75	<b>74</b>	47	46	44	<b>49</b>
Spec, %	93	92	92	<b>92</b>	89	89	87	<b>88</b>	86	86	84	<b>86</b>	95	95	95	<b>94</b>
PPV, %	65	63	60	<b>63</b>	59	59	57	<b>58</b>	56	56	54	<b>56</b>	72	68	68	<b>68</b>
NPV, %	89	88	88	<b>90</b>	91	91	92	<b>91</b>	93	92	93	<b>93</b>	88	88	87	<b>88</b>

Tg, thyroglobulin antibody; TPO, thyroperoxidase antibody; TSH-R, thyroid stimulating hormone receptor antibody; Sens, sensitivity;

Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; Dx, diagnosis

**Supplement Table 5.** Mean Median (Min – Max) antibody levels\* at serum collection time points by case status.

	Graves disease cases (n=87)			Hashimoto thyroiditis cases (n=87)			Controls (n=348)		
	Tg	TPO	TSH-R	Tg	TPO	TSH-R	Tg	TPO	TSH-R
Diagnostic (+/- 6 months of index date)	199.8 72.7 7.8-1617.3	598.7 334.9 0 -1974.8	7.6 3.8 0.1 – 45.8	398.5 129.0 6.4-1959.6	776.1 796.5 0-2300.3	NM	47.7 16.0 7.2-1084.3	91.7 0 0-1761.4	0.4 0.5 0.1 – 0.7
Pre-1 (-6 months to -2 years)	164.8 47.9 7.0-1504.1	418.4 95.2 0-1714.5	2.0 0.7 0.1 – 15.9	334.1 108.4 8.6-2421.7	746.2 678.2 0-1922.0	NM	50.1 16.9 6.0-1414.4	90.0 0 0-1904.8	NM
Pre-2 (-2 years to -5 years)	117.9 30.5 8.5-1068.7	308.0 23.5 0-1660.1	1.4 0.5 0.1 – 30.5	322.3 100.2 8.9-2707.6	735.8 486.7 0-2006.7	NM	43.0 16.8 4.3-1078.3	85.6 0 0-1910.2	NM
Pre-3 (>5 years before index date)	117.7 26.6 7.4-1831.1	221.2 15.4 0-1692.1	0.5 0.4 0.1 – 6.2	345.2 116.0 10.4-3157.3	696.1 594.3 0-1824.0	0.6 0.5 0.1 – 2.6	45.5 15.9 6.7-1111.7	83.8 0 0-2240.6	0.6 0.5 0.1 – 2.9

\*A positive test result for the thyroglobulin and thyroid peroxidase antibody tests is  $\geq 100$  WHO units. A positive test result for the thyroid stimulating hormone receptor antibody test is  $\geq 3$ .

NM, not measured; Tg, thyroglobulin antibody; TPO, thyroperoxidase antibody; TSH-R, thyroid stimulating hormone receptor antibody