DOI: 10.4274/jcrpe.galenos.2020.2020.0184

Research article

Pre-treatment Neutropenia in Children and Adolescents with Autoimmune Hyperthyroidism

Kaori S. Litao M et al. Neutropenia in Autoimmune Hyperthyroidism

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What is already known on this topic?
Untreated hyperthyroidism can cause neutropenia. The prevalence in adults is reported to range between 14-18%. To our knowledge, there is currently no data on this in children and adolescents.

What does this study add?
This study describes the prevalence of pre-treatment neutropenia in autoimmune hyperthyroidism in children and adolescents. It shows that neutropenia in this population resolves in the short term and does not worsen with thionamides.

Abstract
Objective: Neutropenia can occur from untreated autoimmune hyperthyroidism (AIH) or methimazole (MMI); starting MMI in children and adolescents with AIH and neutropenia could thus be worrisome. We aimed to describe the prevalence of neutropenia in children and adolescents with AIH prior to treatment with antithyroid drugs and to assess the effect of antithyroid drugs on the neutrophil count.
Methods: This was a retrospective study of patients with AIH seen at a Pediatric Endocrinology clinic. ANC data at presentation and during anti-thyroid treatment for up to 24 weeks was collected. AIH was defined as elevated fT4 or fT3, suppressed TSH, and positive thyroid autoantibodies. Neutropenia was defined as ANC <1500 cells/µl.
Results: 31 patients were included: 71% females, 29% males, median age 14.71 years (IQR 11.89-17.10). Neither fT4 nor fT3 levels significantly correlated with ANC at presentation ($r_s = 0.22$, $p = 0.24$ and $r_s = 0.13$, $p = 0.54$, respectively). 26/31 (84%) had normal baseline ANC; none developed neutropenia with thionamides. 5/31 (16%) had baseline neutropenia (median 1,200/µl; IQR 874-1200); 4/5 patients started MMI at diagnosis; 1/5 started propranolol only then added MMI 1 week later; all normalized ANC within 24 weeks.
Conclusion: A small percentage of AIH patients may have neutropenia at presentation, but it resolves in the short term and does not worsen with thionamides. Thionamides may be used with caution in these patients with close monitoring of blood counts.
Keywords: Hyperthyroidism, neutropenia, thionamides, methimazole, agranulocytosis

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Submitted: 29.07.2020
Accept: 12.11.2020

Introduction
Autoimmune hyperthyroidism (AIH) is the most common cause of hyperthyroidism in the pediatric population, and methimazole (MMI) the anti-thyroid drug of choice. [1] Agranulocytosis is a known rare but serious adverse reaction to anti-thyroid drugs (ATD), and has been reported to occur in 0.1-0.5% of patients with Graves' disease after initiation of therapy. [2] However, untreated hyperthyroidism itself can cause hematologic abnormalities, including anemia, leukopenia, thrombocytopenia, or rarely pancytopenia; this has been reported several times in the literature, generally in the adult population. Most of these patients were treated with thionamides, with blood counts improving after achievement of euthyroidism. [3-10] In particular, the presence of baseline neutropenia in a patient presenting with hyperthyroidism could be worrisome for the clinician, given the concern for potential agranulocytosis as a side effect of ATDs.
A prospective study by Aggarwal et al found that among newly diagnosed adult patients with Graves disease (n = 206), 14.1% had pre-treatment neutropenia. The study also found that neutrophil counts increased after treatment with antithyroid drugs and that this was related to a reduction in thyroid hormone levels. [6] To our knowledge, there is currently no pediatric data on this subject.
Objectives
The objectives of this study were to describe the prevalence of neutropenia in children and adolescents with AIH prior to treatment with antithyroid drugs and to assess the effect of antithyroid drugs on the neutrophil count.
Methods

This was a retrospective study of patients diagnosed with AIH who were seen at the Pediatric Endocrinology clinic from January 1, 2005 to May 31, 2019. Inclusion criteria were those who have never received ATD, those who had been off ATD for more than 3 months due to non-compliance, those who succeeded initially in being tried off ATD for more than 3 months but went into relapse afterwards, and those who underwent radioactive iodine therapy or surgical thyroidectomy but went into relapse at least 3 months after these procedures. Group A was the control group (i.e., those who were not found to have neutropenia) and Group B consisted of those with neutropenia. Those for whom pretreatment data were unavailable were excluded.

Data up to 24 weeks after treatment was collected. AIH was defined based on elevated free thyroxine (fT4) (N: 0.7-1.5 ng/dL) or free triiodothyronine (fT3) (N: 1.71-3.71 pg/mL), suppressed thyroid stimulating hormone (TSH) (N: 0.4-4.5 mIU/L), and positive thyroid autoantibodies. Neutropenia was defined as ANC <1500 cells/µl [11]. Data collected included patient age, sex, ethnicity, presenting signs and symptoms, levels of TSH, fT4, fT3, anti-thyroglobulin antibody (anti-TG), anti-thyroperoxidase (anti-TPO) antibody, TSH receptor antibody (TRAb), thyroid-stimulating immunoglobulin (TSI), complete blood count (including white blood cell with differential count, red blood cell count, platelet count), as well as levels of anti-neutrophil cytoplasmic antibodies (ANCA). The study protocol was reviewed and approved by the NYU School of Medicine's Institutional Review Board as Exempt Category 4. As this was a retrospective chart review which did not make use of identifiable health information and provided no more than minimal risk to the subjects, informed consent was not necessary, and a request for waiver of authorization to use identifiable health information for research was approved by the NYU School of Medicine's Institutional Review Board in accordance with 45 CFR.164.512.

Statistical Analysis

This was primarily a qualitative study that looked at the prevalence of pre-treatment neutropenia in newly diagnosed patients with pediatric autoimmune hyperthyroidism. Statistical analysis was done to find whether fT4 or fT3 correlated with pre-treatment ANC. None of the variables had normally distributed data, thus a Spearman correlation test was performed using the SciPy library program. Median values as well as the interquartile range (IQR, 25^{th}-75^{th} percentile) for age, fT4, fT3 and ANC were obtained using the same.

Results

A total of 31 patients with AIH were included. Six patients were excluded due to absence of pre-treatment data. All patients were newly diagnosed and had never received ATD. Median age was 14.71 years (IQR 11.89-17.10). 71% were females and 29% were males. 48% were Hispanic (n = 15), 32% Asians (n = 10), 10% Caucasians (n = 3), 6% African Americans (n = 2), and 3% Middle Eastern (n = 1). All had positive thyroid antibodies: 5 (16%) with TRAb/TSI only**,** 4 (13%) with anti-thyroperoxidase (a-TPO) and/or anti-thyroglobulin (a-TG)**,** 9 (29%) with TRAb/TSI and either anti-TPO or anti-TG, and 9 (29%) were positive for all three. 4 (13%) had only TSI measured and were positive. Family history of thyroid disease was present in 15 out of 29 (2 had no data). Neither fT4 nor fT3 levels significantly correlated with ANC at presentation ($r_s = 0.22$, $p = 0.24$ and $r_s = 0.13$, $p = 0.54$, respectively). (Table 1, Figures 1 and 2)

26 out of 31 patients (Group A, 84%) had normal baseline ANC (median 3,800/µl; IQR 2,925-5,200). 77% were females and 23% were males. Median fT4 was 3.47 ng/dL (IQR 2.63-5.84) and fT3 12.2 pg/mL (IQR 8.25-18.4) (n = 23). None of these patients developed neutropenia after starting thionamides.

5 out of 31 patients (Group B, 16%) had baseline neutropenia (median 1,200/µl; IQR 874-1200). 40% were females and 60% were males. Median fT4 was 1.92 ng/dL (IQR 1.79-3.2) and fT3 8.3 pg/mL (IQR 7.2-13.65) (n = 3). In 4/5 patients, MMI was started at diagnosis; 1/5 was started on propranolol only followed by MMI 1 week later. All 5 cases normalized their ANC within 24 weeks. 4/5 cases had normal ANC by 4 weeks (1 patient developed a transient drop in ANC at week 9 which again normalized at 21 weeks). 1/5 continued to have neutropenia at 12 weeks and normalized by 24 weeks. In addition, 3/5 patients had anti-neutrophil cytoplasmic antibodies checked and were negative. (Table 2 and Fig. 1)

Discussion

In this study, 16% of pediatric patients with AIH were found to have neutropenia prior to initiating treatment with ATDs, similar to studies in adults which have reported prevalences between 14-18%. [6, 12] Most of our patients who had pre-treatment neutropenia (60%, 3/5 patients) had ANC >1000 cells/µl; one had moderate neutropenia (874 cells/µl) and one had severe neutropenia (400 cells/µl). In an adult study by Aggarwal et al, 14.1% of patients with Graves disease (n = 206) had pretreatment neutropenia, although the study used an ANC cutoff of <2000 cells/µl; mean ANC in these patients was 1600 ± 300 cells/µl. The study found that neutrophil counts in patients with pre-treatment neutropenia increased after initiation of ATDs; euthyroidism in these patients was achieved after a median time period of 55 days, and the increase in ANC was found to be related to a reduction in thyroid hormone levels. [6] In our study, neither fT4 nor fT3 levels were found to correlate with ANC at baseline. Although Aggarwal et al used an ANC cutoff of <2000 cells/µl, in pediatrics, neutropenia is conventionally defined as an ANC <1500 cells/µl, so we opted to use this cutoff in our study. [11] Gangadharan et al reported a case of a 13 year old boy with Graves' disease and a pre-treatment ANC of <1500 cells/µl; the child was treated with propranolol with Lugol's iodine; neutrophil counts improved after 16 days of treatment, upon which carbimazole was started. [13]

Neutropenia has been reported to occur in 0.02 to 0.04% of children with Graves' disease upon starting MMI [14, 15]. In a study by Rabon et al, the pediatric patients who developed neutropenia after starting MMI (9 out of 251) had mild neutropenia, with none having an ANC <1000 cells/µl. [15] Rivkees et al studied 100 consecutively treated pediatric patients with Graves' disease, of which 2 developed moderate neutropenia (500 and 750 cells/µl). [14] These studies looked at the overall rates of adverse events with MMI and did not go into particulars as to how the patients with neutropenia were managed, but generally, the patients who developed adverse effects with MMI stopped the medication and underwent definitive therapy, i.e., surgery or radioactive iodine. Methimazole-associated agranulocytosis is thought to be due to either direct toxicity when the drug is oxidized by neutrophils to reactive metabolites, or to immune-mediated mechanisms (e.g., the presence of anti-neutrophil

cytoplasmic antibodies); these are generally believed to be idiosyncratic reactions. [16-18] However, a large study by Takata et al found that agranulocytosis was significantly more common in those who received a higher daily dose of MMI (0.8% in those who received 30 mg, n = 2087; 0.3% in those who received 15 mg, n = 2739; p<0.001), suggesting that there could be a dose-dependent mechanism as well. In our study, none of the patients who had a normal ANC at baseline (Group A) developed neutropenia after treatment with thionamides. Patients who had pre-treatment neutropenia (Group B) were started on standard doses of methimazole (Table 2), and none of them developed severe agranulocytosis with treatment.

Several studies have reported not only neutropenia but pancytopenia as a complication of poorly controlled Graves' disease [3-5, 7, 8, 10, 19-23] which responded to ATDs with or without RAI. In one case report [10], although pancytopenia improved with ATDs, there was a recurrent increase in thyroid hormone levels and pancytopenia after 4 months, upon which subtotal thyroidectomy was performed. The patient's pancytopenia then resolved with good control of thyroid function.

The mechanism for pre-treatment hyperthyroidism-associated neutropenia has not been completely elucidated, but Kyritsi et al found that out of 218 adult patients who presented to a hematology clinic with neutropenia, 43.6% had thyroid disease (including Graves' disease, Hashimoto's thyroiditis, patients who had undergone total thyroidectomy, nontoxic multinodular goiter, and antibody-negative subclinical hypothyroidism). Although patients who were undergoing treatment with ATDs were not excluded from the study, the authors did find that there was an inverse correlation between free T3 and the ANC (r^2 = -0.274, p= 0.007), suggesting a direct toxic effect of excess thyroid hormone to granulopoiesis. This is further supported by the fact that the subgroup of patients (n = 6) who had total thyroidectomy and subsequent iatrogenic medication-related hyperthyroidism had the lowest ANC, whereas those with non-toxic multinodular goiter (n= 18), who were euthyroid with no detectable antithyroid antibodies, had the highest ANC. In the same study, it was also found that CD4+ lymphocytes positively correlated with TSH levels (r^2 = 0.16, p = 0.045), but negatively with T4 levels (r^2 = -0.274, p = 0.024). [24] The possible involvement of autoimmune anti-neutrophil antibodies causing hyperthyroidism-associated neutropenia has also been postulated. [4, 8] In our study, 3 out of the 5 patients who had pre-treatment neutropenia had anti-neutrophil cytoplasmic antibodies (ANCA) checked and were negative. ANCA has been associated with autoimmune neutropenia, albeit a direct causal relationship has not been established. ANCA has typically been associated with vasculitides, but neutrophil destruction by antineutrophil membrane antibodies may expose PR3 and MPO antigens to the circulation, thereby promoting ANCA formation. It has been suggested that a positive ANCA should prompt one to think of underlying toxin exposure or other autoimmune diseases. [25] Methimazole-associated agranulocytosis is considered a form of transient acquired neutropenia (TAN) defined as neutropenia lasting <3 months. The differential diagnoses for TAN include infection (especially viral infections), autoimmune neutropenia and drug-induced neutropenia (mainly anticonvulsants, sulfonamides, penicillins, antipsychotics and ATDs). Recombinant granulocyte colony stimulating factor is typically only used in these cases if the neutropenia is profound and associated with severe infection. [11]

Thyrotoxicosis results in an increased response to catecholamines, particularly beta-adrenergic signaling, causing signs and symptoms such as palpitations, hypertension, and weight loss. The effects of thyroid hormone on alpha-adrenergic signaling is less clear. [26] Increased beta-adrenergic activity as a result of thyrotoxicosis is less likely to be the cause of neutropenia since studies have shown that beta-adrenergic effects on the bone marrow is actually to increase neutrophil production. [27]

Although limited by its retrospective nature, small sample size, and short duration of follow-up, our study found that the patients with AIH and pre-treatment neutropenia did not have worsening neutropenia with thionamides. Larger longitudinal studies would be of benefit to elucidate if and how the thyrotoxic state or autoimmune factors correlate with ANC.

Conclusion

A small percentage of patients with AIH may have pre-treatment neutropenia, but it resolves in the short term and does not worsen with thionamides. Anti-thyroid drugs may be used with caution in these patients with close monitoring of blood counts.

Authorship Contribution

MKSL primarily wrote the entire article and formatted the tables. AGA reviewed and edited the manuscript and provided additional interpretation of results. BS assisted with writing the article, reviewed and edited the manuscript, and provided additional interpretation of results.

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Table 1: Baseline characteristics of patients with autoimmune hyperthyroidism
Median (IQR) or n (%)

	n	Total	Group A n = 26 (84%)	Group B n = 5 (16%)
Age in yrs	31	14.71 (11.89-17.10)	14.6 (11.02-16.90)	16.58 (14.75-18.58)
Sex				
Males	31	9 (29%)	6 (23%)	3 (60%)
Females		22 (71%)	20 (77%)	2 (40%)
Ethnicity				
Hispanic	31	15 (48%)	14 (54%)	1 (20%)
Asian		10 (32%)	7 (27%)	3 (60%)
Caucasian		3 (10%)	3 (12%)	0
African American		2 (6%)	1 (4%)	1 (20%)
Middle Eastern		1 (3%)	1 (4%)	0
Thyroid antibodies				
TRAb/TSI only	31	5 (16%)	4 (15%)	1 (20%)
anti-TPO or anti-TG only		4 (13%)	3 (12%)	1 (20%)
TRAb/TSI + anti-TPO or anti-TG		9 (29%)	8 (31%)	1 (20%)
All three		9 (29%)	8 (31%)	1 (20%)
Only TSI measured (positive)		4 (13%)	3 (12%)	1 (20%)
Family history of thyroid disease	29	15 (52%)	13/24 (54%)	2/5 (40%)
fT4 (N: 0.7-1.5 ng/dL)	31	3.20 (2.22-5.59)	3.47 (2.63-5.84)	1.92 (1.79-3.2)
fT3 (N: 1.71-3.71 pg/mL)	26	11.60 (8.22-18.70)	12.2 (8.25-18.4)*	8.3 (7.2-13.65)**
ANC (cells/µl)	31	3,400 (2,400-4,350)	3,800 (2,925-5,200)	1,200 (874-1,200)

**Group A: 23/26 patients had fT3 levels*
***Group B: 3/5 patients had fT3 levels*

Table 2: Characteristics, treatment regimen, and ANC response in those with pre-treatment neutropenia

	Age (yrs), sex	Ethnicity	Baseline ANC (cells/uL)	Baseline fT4 (ng/dL)	Baseline fT3 (pg/mL)	Treatment		ANC response				
						MMI (mg daily dose)	propranolol (mg TID)	2-4 wks	4-8 wks	8-12 wks	12-20 wks	20-24 wks
Patient 1	18.6, M	A	1458	1.4	-	5*	-	-	1900	1800	1900	1500
Patient 2	16.6, M	AA	874	3.2	>19	20	20	1800	1500	1300	1700	-
Patient 3	4.8, F	A	400	1.79	6.1	2.5**	1.25**	4170	3850	5370	2270	-
Patient 4	18.7, M	H	1200	1.92	8.3	20	-	-	-	1400	-	1700
Patient 5	14.7, F	A	1200	3.55	-	30	-	2600	3800	2500	-	-

A: Asian, AA: African American, H: Hispanic
**MMI x 1 month then PTU 100 mg BID*
***propranolol monotherapy x 1 week before adding MMI*

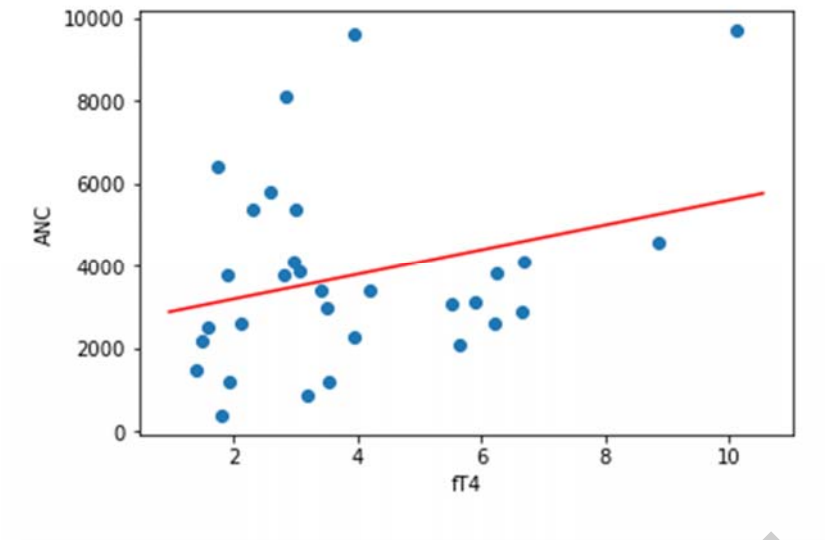

Figure 1: Lack of correlation between pre-treatment fT4 and ANC

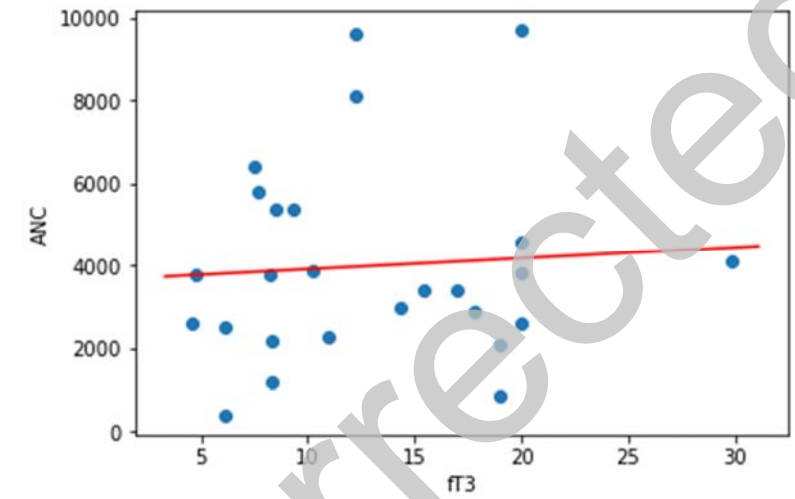

Figure 2: Lack of correlation between pre-treatment fT3 and ANC