



Prevalence of Anti-Gastric Parietal Cell Antibodies and Vitamin B12 Deficiency in Rheumatoid Arthritis

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Abstract

Recent studies are lacking in assessing presence of both the anti-gastric parietal cell (GPC) antibody and concurrent vitamin B12 deficiency in Rheumatoid Arthritis (RA) patients in the era of more aggressive disease modifying therapy. We recruited patients to one of three arms: those with RA, autoimmune thyroid disease (AITD), and no known autoimmune disorder (controls). A one-time serum assessment of vitamin B12 level, methylmalonic acid, and anti-GPC antibody level was performed. Forty-five subjects enrolled in the RA arm, 36 in the AITD arm, and 44 controls. Levels of anti-GPC antibodies were not statistically different. Serum vitamin B12 levels were not statistically different across the three groups. Only 2 subjects were vitamin B12 deficient requiring therapy, none in the RA arm.

Keywords

Rheumatoid Arthritis, Vitamin B12 deficiency, Gastric parietal Cell antibody, Disease modifying therapy

Introduction

Rheumatoid Arthritis (RA) is a chronic systemic inflammatory and immune-mediated disease with both articular and extra-articular manifestations. It manifests with synovial inflammation, autoantibody production, cartilage and bone destruction, and systemic features such as accelerated cardiovascular disease and osteoporosis. A hallmark of RA as well as a finding of diagnostic and prognostic importance is the presence of autoantibodies. Two well described autoantibodies associated with RA are the rheumatoid factor (RF) and anti-citrullinated protein antibody (detected using an anti-cyclic citrullinated peptide (anti-CCP) IgG assay). These autoantibodies can be often be detected in patients before the development of arthritis. Production can be stimulated by infection (response of RF which has a high-affinity against the Fc portion of the immunoglobulin) or by various cytokines (interleukin-6 propagates leukocyte activation and autoantibody formation) [1].

The prevalence of organ specific autoimmune conditions appears to be increased in patients with RA. For example, over the last few decades an increased occurrence of thyroid disorders in patients with RA has been reported. This association has both autoimmune and non-autoimmune links [2]. The prevalence of both Hashimoto's Thyroiditis and Graves' disease are significantly higher in RA patients as compared to the general population [3]. Other organ specific

autoimmune diseases more prevalent in RA patients as compared to the general population include vitiligo [4], myasthenia gravis, and others [5,6] Environmental triggers in a genetically susceptible host, specifically those with the human leukocyte antigen (HLA)-B8, DR3, or DR5, likely lead to loss of self-tolerance and other abnormalities in both humoral and cell-mediated immunity [5].

Pernicious anemia, a manifestation of vitamin B12 deficiency, is an autoimmune gastritis which results from the destruction of gastric parietal cells and the subsequent loss of intrinsic factor (IF) to bind dietary vitamin B12. In pernicious anemia the immune response is focused on the gastric H/K ATPase [7]. In some cases, an autoantibody directed against IF can be detected. P anemia appears to be more prevalent in RA, a relationship noted since the mid-to-late 1960s [8]. Over the course of several decades, few studies have assessed the potential co-existence of vitamin B12 deficiency due to gastric parietal cell autoantibodies. While Vreugdenhil et al. in 1990 and Segal et al. in 2004 published studies which found that 29% and 49% of patients with RA had vitamin B12 deficiency, no assessment of the etiology or the presence of autoantibodies was made [9,10]. While Goeldner et al. in 2011 and Datta et al. in 1990 demonstrated that anti-gastric parietal cell antibodies (anti-GPC Ab) were found in < 5% to 28% of RA patients respectively, no additional testing was undertaken to determine a relationship with vitamin B12 deficiency [11,12].

Over the last two decades, the management of RA has changed significantly. More aggressive and effective use of disease modifying anti-rheumatic drugs (DMARDs) and biologic response modifiers (BRMs) make it possible for a majority of patients to achieve disease remission. Severe disease manifestations such as vasculitis, nodulosis, scleritis, and amyloidosis more common with persistent and suboptimally controlled inflammation, have become rare [1]. Routine use of some biologic response modifiers also alters the autoantibody milieu, decreasing autoantibodies such as the anti-CCP IgG and RF [13]. This study tested the hypothesis that given these recent improvements in multiple facets of RA treatment, the prevalence of vitamin B12 deficiency along with anti-GPC Ab positivity was declining. We also wanted to determine the prevalence of those patients with RA who had a positive anti-GPC Ab test who were also vitamin B12 deficient, as this relationship would raise awareness amongst clinicians to assess their patients clinically or serologically for vitamin B12 deficiency.

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Methods

This cross sectional study was performed at a single academic community hospital from April 2013 to September 2014. Subjects were recruited from internal medicine (IM) and IM Specialties Clinic, specifically the Endocrinology and Rheumatology Clinics. Data collection, record reviews, and patient interviews were performed by the study investigators.

Subjects were enrolled in one of three groups. The first group was a group with RA. RA was diagnosed by a board certified, actively practicing Rheumatologist based on clinical, serologic, and/or radiographic findings that a subject presented with consistent with the 1987 or 2010 American College of Rheumatology Classification Criteria for RA (depending on the year when they were diagnosed). Seronegative RA subjects had either extra-articular manifestations typical for RA, radiographic or magnetic resonance imaging (MRI) of the hands which demonstrated synovial inflammation or erosions consistent with a polyarticular symmetric small joint arthritis favoring RA, or were diagnosed by a different rheumatologist with RA. The second group was an autoimmune thyroid disease (AITD) group. Subjects were considered to have AITD if they had a history of hypothyroidism or hyperthyroidism not attributable to a non-immune cause (such as hypothyroidism post-thyroidectomy for thyroid cancer). The purpose of the AITD arm was that it would represent a cohort of subjects with an autoimmune disease which can also be associated with other organ specific or systemic autoimmune illnesses and subjects with AITD would not typically be treated with DMARDs or BRMs for this condition. The last group was a control group. These subjects had no known history and no symptoms or other clinical findings consistent with RA or AITD.

The study was open to all adults 18 years of age or older. Subjects were excluded if they had a known history of vitamin B12 deficiency, regardless of how it was diagnosed. Subjects were excluded if they reported symptoms or had complete blood count findings (such as an elevated mean corpuscle volume or macrocytosis) suspicious for vitamin B12 deficiency. Subjects were also excluded if they had a known medical or surgical malabsorptive state such as celiac disease, inflammatory bowel disease, gastric bypass, short bowel syndrome, or other intestinal surgery.

At the time of enrollment, subjects completed a one-page questionnaire soliciting use of proton pump inhibitors (such as omeperazole), over the counter or prescription vitamin use, vegan/vegetarian dietary habits, metformin use, and the use of disease modifying anti-rheumatic drug (DMARD) and/or biologic response modifier use. Subjects then underwent one-time blood draw. Vitamin B12 and Methylmalonic acid (MMA) level were determined by electro-chemiluminescence immunoassay (ECLIA) and liquid chromatography. Anti-thyroid peroxidase (TPO), anti-thyroid stimulating hormone receptor (TSHR) Ab, and anti-GPC antibodies were determined by ECLIA and enzyme linked immunosorbent assay (ELISA). RF and anti-CCP IgG levels were determined by latex immunoturbidimetry and immunoassay respectively.

The primary outcome of the study was to determine the prevalence of anti-GPC antibodies and prevalence of serum vitamin B12 deficiency as measured by either a low vitamin B12 level and/or elevated MMA in subjects with RA receiving DMARDs and/or BRMs for their inflammatory arthritis. With the Type I error set at 0.05, a power of 0.8, and an estimated effect size of 0.3, we calculated that we would need to recruit 132 subjects with 44 in each of the 3 groups. Differences in continuous variables were assessed by ANOVA. Differences in categorical variables assessed by Chi squared testing or likelihood ratios. Statistical analysis was performed using SPSS (version 22; SPSS Inc, Chicago, IL, USA). Written informed consent was obtained from all subjects prior to enrollment and serologic testing. The study was approved by the hospital Institutional Review Board before data collection began. This trial was registered with www.clinicaltrials.gov (NCT01876329).

Table 1: Demographics.

	RA (n = 45)	AITD (n = 36)	Control (n = 44)
Age (\pm StDev)	59.6 \pm 13.1	55.1 \pm 17.2	55.8 \pm 16.4
Male : Female	11 : 34	6 : 30	13 : 31
Ethnicity (%)			
Caucasian	73.3%	77.8%	77.3%
African American	22.2%	8.3%	15.9%
Asian	2.2%	5.6%	4.5%
Pacific Islander	2.2%	0%	0%
Hispanic	0%	2.8%	0%
Other	0%	5.6%	2.3%

Key: StDev – Standard Deviation

Results

One-hundred and twenty seven subjects were enrolled but two subjects were excluded from the final data analysis. Both subjects excluded were enrolled in the RA group: one subject had symptoms at the time of enrollment suspicious for (and later confirmed to be attributable to) inflammatory bowel disease and one subject was inadvertently enrolled who had neuropathic symptoms and was undergoing investigation for vitamin B12 deficiency. Of the 125 subjects for whom data was analyzed, 45 were in the RA group, 36 were in the AITD group, and 44 were controls. The demographics of the 3 groups are shown in table 1. There were no statistically significant differences observed amongst the three groups regarding age, gender, or ethnicity.

In the RA group, 80.0% were seropositive and 20.0% were seronegative. At the time of enrollment, 42 (92.3%) were actively receiving therapy with DMARDs and/or BRMs. Nearly all (97.8%) subjects in the RA group were treated at some time with DMARDs and/or BRMs. Eight (17.8%) subjects in the RA group had a concomitant diagnosis of hypothyroidism and were actively receiving Synthroid; all 8 were female and 7 had AITD with one having a surgically treated multinodular goiter.

In the AITD group, all 36 subjects were prescribed levothyroxine therapy and were clinically and biochemically euthyroid. At the time of enrollment, 2 of the subjects were actively being treated with DMARDs (one for psoriasis and one for dermatomyositis) and 2 were actively being treated with BRMs (both for psoriasis). Twenty-five (69%) had a history of hypothyroidism and 8 of these were anti-TPO Ab positive. Eleven (31%) of the AITD group had a history of treated Graves' disease, with 7 of these subjects anti-TSHR Ab positive. In the control group, 8 of the 44 subjects were actively being treated with either a DMARD (1) or BRM (7).

There were no statistically significant differences observed in current proton pump inhibitor use, previous proton pump inhibitor use, number of proton pump inhibitors used, or metformin use amongst the three groups in this study. Vitamin B12 supplementation was equal across the three groups. Subjects in the RA groups were on proton pump inhibitors longer than those in the AITD group ($p = 0.016$) and control group ($p = 0.003$).

The level of anti-GPC Ab amongst the three groups is shown in table 2, along with other autoantibodies such as anti-TPO Ab, anti-nuclear antibody titer over 1:80, anti-CCP IgG levels, and RF positivity. As expected, anti-CCP IgG positivity and RF positivity were statistically more likely to have occurred in the RA group (both $p < 0.001$). There were no differences in any of the three groups regarding differences in anti-GPC Ab, anti-TPO Ab, or anti-nuclear antibody titers over 1:80.

The serum level of vitamin B12 and methylmalonic acid along with new cases of vitamin B12 deficiency based on several case definitions are noted in table 3. There were no statistically significant differences in vitamin B12 or methylmalonic acid levels across the three groups. There were also no statistically significant differences in new cases of vitamin B12 detected across the three groups, regardless of serum vitamin B12 cutoff used (< 200 pg/mL or < 350 pg/mL) or

Table 2: Anti-GPC Ab levels and other autoantibodies.

	RA (n = 45)	AITD (n = 36)	Control (n = 44)	'p' value
Number of Anti-GPC Ab > 24.9 Units* (Prevalence %)	7 (15.6%)	4 (11.1%)	8 (18.2%)	
Anti-GPC Ab levels (Units)	23.7 ± 18.8	18.3 ± 22.2	22.6 ± 25.5	0.538
Number of Anti-TPO Ab > 34 International Units/mL (Prevalence %)	4 (8.9%)	12 (33.3%)	7 (15.9%)	
Anti-TPO Ab levels (International Units/mL)	15.1 ± 11.1	287.5 ± 949.5	46.4 ± 146.1	0.087
Anti-nuclear antibody titer over 1:80	8	1	2	
Anti-CCP IgG levels (Units)	145.1 ± 103.7	11.1 ± 5.6	11.5 ± 7.6	< 0.001
Number of RF > 15 International Units/mL (Prevalence %)	31 (68.9%)	1 (2.8%)	4 (9.0%)	
RF value (International Units/mL)	73.0 ± 71.0	10.0 ± 4.2	14.4 ± 20.9	< 0.001

Key: *Threshold at which anti-GPC or anti-TPO antibodies as well as rheumatoid factor (RF) were counted as "positive" were determined by established ranges provided by our reference lab.

Table 3: Vitamin B12 levels, Methylmalonic acid levels, and vitamin B12 deficiency.

	RA (n = 45)	AITD (n = 36)	Control (n = 44)	'p' value
Vitamin B12 levels (pg/mL)	726.3 ± 392.9	606.8 ± 281.8	655.8 ± 342.7	0.297
Methylmalonic acid levels (nmol/L)	202.4 ± 79.3	216.1 ± 125.1	190.5 ± 118.4	0.575
Vitamin B12 < 200 pg/mL (Prevalence %)	0 (0%)	0 (0%)	1 (2.3%)	NS
Vitamin B12 < 350 pg/mL (Prevalence %)	3 (6.7%)	6 (16.7%)	4 (9.1%)	NS
Vitamin B12 < 220 pmol/L AND Methylmalonic acid > 378 nmol/L* (Prevalence %)	0 (0%)	1 (2.8%)	1 (2.3%)	NS

Key: *Marks PW, et al. NEJM. 2004; 351: 1338.

The one subject in the control arm with a serum vitamin B12 level less than 200 pg/mL was the same subject who also had a serum vitamin B12 < 220 pmol/L and Methylmalonic acid > 378 nmol/L

To convert values for serum vitamin B12 from picograms per milliliter to picomoles per liter, multiply by 0.7378.

combination of serum vitamin B12 and methylmalonic acid level used [14]. No subjects in the RA had serologic evidence of vitamin B12 deficiency. One subject in the AITD group was identified as having vitamin B12 deficiency as he had a serum vitamin B12 < 220 pmol/L and Methylmalonic acid > 378 nmol/L [14]. One subject in the control arm was also identified as having vitamin B12 deficiency as he had a serum vitamin B12 level less than 200 pg/mL and also had a serum vitamin B12 < 220 pmol/L and Methylmalonic acid > 378 nmol/L [14].

Finally, subjects were grouped based on whether or not they reported consumption of an over-the-counter vitamin B12 or any vitamin supplement. Those that reported consumption had a serum vitamin B12 level of 842.7 ± 376.4 pg/mL whereas those that did not report consumption of vitamin supplements had a serum vitamin B12 level of 606.6 ± 316.1 pg/mL (p = 0.001).

Discussion

Rheumatoid Arthritis as an illness has dramatically changed over the last 25 years. Aggressive use of DMARDs and BRMs had made disease remission possible and severe disease manifestations rare [1]. Some BRMs also alters the autoantibody milieu, decreasing autoantibodies such as the anti-CCP IgG and RF [13]. We designed this study to determine if similar changes were occurring with regard to the production of anti-GPC Ab and prevalence of vitamin B12 deficiency. Our cross sectional study design assessed subjects with RA and AITD across a spectrum of disease duration, presence or absence of autoantibodies, and possible DMARD/BRM use that would be routinely encountered in a clinical setting. We also tried to account for contemporary trends in dietary habits (vegetarianism and veganism) as well as medication use, specifically use of proton pump inhibitors and metformin, as all of these could impair vitamin B12 absorption [15]. Our data suggest that the presence of anti-GPC Ab and vitamin B12 deficiency may not be as common in our study population, although the wide range of previous studies make it somewhat challenging to interpret any trends. A factor that likely contributes to this is defining vitamin B12 deficiency. Using several different definitions, we obtained several different prevalence rates (Table 3). Though not statistically significant, it is interesting to note that the AITD arm had a higher prevalence of vitamin B12 deficiency, although this was not statistically significant. Whether or not these findings relate to recent improvements in RA disease control remains unclear.

Our study has several weaknesses which limit the generalizability

of our data. First, with the low prevalence of both vitamin B12 deficiency and anti-GPC Ab identified in RA arm, our study was underpowered at the time of data analysis. With 125 subjects recruited, the observed effect size for differences in serum vitamin B12 level was 0.14. This yielded a power of 0.26. The observed effect size for differences in anti-GPC Ab was 0.10, which yielded a power of 0.15. Data analysis was performed as we were close to our anticipated pre-study recruitment goal, however given the small effect size observed, we estimated that over 500 subjects total would need to be recruited. Second, we suboptimally accounted for the moderate effect size that vitamin B12 supplementation would have on our study results. Given contemporary over-the-counter vitamin trends, those supplementing with vitamin B12 had a statistically significant 238 ng/mL higher level than those not taking vitamin B12 supplements. The power of this relationship was 0.91, thus the moderate effect size of vitamin B12 supplementation makes it difficult to detect smaller differences that might actually be due to anti-GPC Ab and vitamin B12 deficiency. Third, the inclusion of subjects on DMARDs or BRMs in an arm other than for RA likely introduced heterogeneity into the AITD and control arms as our premise was that aggressive disease control of RA with these medications shape the autoantibody milieu. We debated removing subjects in the AITD or control arm who were on a DMARD and/or BRM from our final analysis, but we thought this exclusion would introduce more bias. Finally, it is possible that autoantibodies to IF have not changed in prevalence or have increased, but since we did not study this autoantibody, temporal trends in this specific autoantibody and how it relates to vitamin B12 deficiency was potentially missed. It would seem from the low prevalence of anti-gastric parietal cell antibodies and vitamin B12 deficiency observed in this study that screening for an autoantibody to IF would be of limited value.

In conclusion, the intent of this study was to study the change in anti-GPC Ab levels and vitamin B12 deficiency over the last decade or so as higher rates of anti-GPC Ab and vitamin B12 deficiency appear to have been observed in the years before aggressive RA disease control with DMARDs and/or BRMs was the norm. While we attempted to account for over-the-counter vitamin trends as well as proton pump inhibitor and metformin use, we fell short in understanding the moderate effect size that current subject vitamin B12 use would have.

Reference

1. Mc Innes IB, Schett G (2011) The pathogenesis of rheumatoid arthritis. N Engl J Med 365: 2205-2219.

2. Cárdenas Roldán J, Amaya-Amaya J, Castellanos-de la Hoz J, Giraldo-Villamil J, Montoya-Ortiz G, et al. (2012) Autoimmune thyroid disease in rheumatoid arthritis: a global perspective. *Arthritis* 2012: 864-907.
3. Biró E, Szekanez Z, Cziráj L, Dankó K, Kiss E, Szabó NA, et al. (2006) Association of systemic and thyroid autoimmune diseases. *Clin Rheumatol* 25: 240-245.
4. Michou L, Rat AC, Lasbleiz S, Bardin T, Cornélis F (2008) Prevalence and distribution of autoimmune diseases in 368 rheumatoid arthritis families. *J Rheumatol* 35: 790-796.
5. Melikoğlu MA, Melikoğlu M, Karatay S, Uğur M, Şenel K (2008) A Coincidence of Rheumatoid Arthritis, Autoimmune Thyroid Disease and Vitiligo in a Single Patient: A Possible Pathogenetic Linkage. *Eurasian J Med* 40: 42-44.
6. Teufel A, Weinmann A, Kahaly GJ, Centner C, Piendl A, et al. (2010) Concurrent autoimmune diseases in patients with autoimmune hepatitis. *J Clin Gastroenterol* 44: 208-213.
7. Stabler SP (2013) Vitamin B12 deficiency. *N Engl J Med* 368: 2041-2042.
8. Whittingham SF, Mackay IR (1967) Pernicious anemia and Rheumatoid Arthritis. *Arthritis Rheum* 10: 259-261.
9. Vreugdenhil G, Wognum AW, van Eijk HG, Swaak AJ (1990) Anaemia in rheumatoid arthritis: the role of iron, vitamin B12, and folic acid deficiency, and erythropoietin responsiveness. *Annals of the Rheumatic Diseases* 49: 93-98.
10. Segal R, Baumoehl Y, Elkayam O, Levartovsky D, Litinsky I, et al. (2004) Anemia, serum vitamin B12, and folic acid in patients with rheumatoid arthritis, psoriatic arthritis, and systemic lupus erythematosus. *Rheumatol Int* 24: 14-19.
11. Goeldner I, Skare TL, de Messias Reason IT, Nishihara RM, Silva MB, et al. (2011) Autoantibodies for gastrointestinal organ-specific autoimmune diseases in rheumatoid arthritis patients and their relatives. *Clin Rheumatol* 30: 99-102.
12. Datta A, Deodhar SD, Datta U, Sehgal S (1990) Non-organ specific & organ specific antibodies in rheumatoid arthritis. *Indian J Med Res* 92: 228-232.
13. Modi S, Soejima M, Levesque MC (2013) The effect of targeted rheumatoid arthritis therapies on anti-citrullinated protein autoantibody levels and B cell responses. *Clin Exp Immunol* 173: 8-17.
14. Marks PW, Zukerberg LR (2004) Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 30-2004. A 37-year-old woman with paresthesias of the arms and legs. *N Engl J Med* 351: 1333-1341.
15. Niafar M, Hai F, Porhomayon J, Nader ND (2015) The role of metformin on vitamin B12 deficiency: a meta-analysis review. *Intern Emerg Med* 10: 93-102.