Clinical Utility of intrinsic factor antibody reflex testing in samples positive for gastric parietal cell antibodies.

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Introduction

Intrinsic factor is secreted by parietal cells that helps absorption of Vitamin B12 in the intestines. The Immunology laboratory employs a 'screening test' using indirect immunofluorescence (IIF) to detect gastric parietal cell (GPC) antibody, followed by reflex EIA test to detect intrinsic factor antibody (IFA) on positive samples in suspected pernicious anaemia. There remains a paucity of guidance on the most reliable testing strategy for diagnosis of pernicious anaemia.

Objectives

The study will assess the utility of the reflex testing method by identifying:

1. Positive rate of detection of GPC,
2. Numbers of reflex IFA tests,
3. How many patients had full blood count and B12 levels checked,
4. Whether this changed clinical management (i.e., B12 replacement therapy instituted).

Methods

Anonymised laboratory data (including age, gender) between 2019-2021 retrospectively studied

Samples stratified on IIF positivity [*see **figure 1***]
Only strong positive samples from primary care were analysed.

We further analysed to see if both IFA and B12 levels were reported.

Correlations between GPC antibody and medical co-morbidities analysed, including how many patients were on regular B12 replacement therapy.

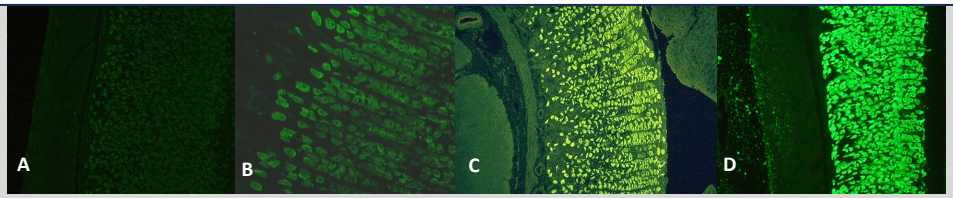

Figure 1: *showing immunofluorescence to detect GPC antibody. A) Negative for GPC antibody, B) weakly positive for GPC antibody, C) Positive for GPC antibody, D) strongly positive GPC antibody*

Results

1. Between the years 2019-2021, 14997 samples reported for GPC antibody status with average 3.2% positive GPC samples per year (mean of 0.82% strongly positive) [See **Table 1** below]. 63 patients (46 females, 17 males) were identified to be strongly positive for GPC and referred from primary care (0.4%).

Year	Total samples tested	Samples positive for GPC antibody						
		Weak positive	Positive	Strong positive	Total +ve samples per year		Strongly +ve/year	
2019	4618	45	58	27	130	2.8%	27	0.6%
2020	3362	30	43	32	105	3.1%	32	1.0%
2021	7017	79	103	65	247	3.5%	65	0.9%
3 years	14997	154	204	124	482	3.2% (average)	124	0.82% (average)

Table 1: Table 1. Yearly breakdown of numbers of samples reported for GPC antibody

2. 57 patients (90.5%) had confirmatory IFA test of whom only 11 patients (17.5%) were positive.
3. 58 patients (92.1%) had full blood count (Hb, MCV) within 3 months of GPC antibody test. 27 patients (42.9%) had B12 level tested, of whom 13 patients (48.1%) were found to have level lower than the reference range (115-1000 pmol/L).
4. 36 patients (59.0%) did not receive B12 therapy. Of those receiving B12 therapy, 10 were positive for IFA. Medical co-morbidities were identified in 61 patients (See **Figure 2** pie chart).

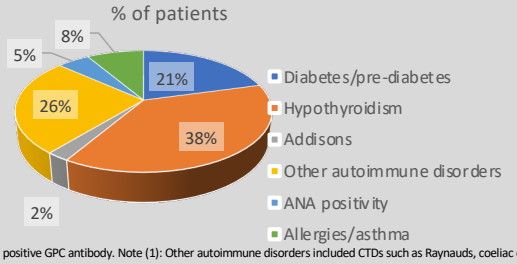

Figure 2. Medical co-morbidities identified in 61 patients with strongly positive GPC antibody. Note (1): Other autoimmune disorders included CTDs such as Raynauds, coeliac disease, Sjogrens and psoriasis; note (2): some patients in the table are included more than once due to the patient's medical history

Discussion

Continuous B12 therapy was provided to 25 patients, [see **figure 3**].

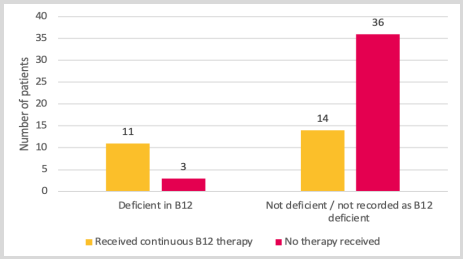

Figure 3 : Bar Graph showing breakdown of B12 deficiency and B12 replacement therapy being instituted

10 IFA positive patients were commenced on B12 therapy, of which 4 were deficient in B12. Comparatively, irrespective of B12 levels, of 52 patients strongly positive for GPC and negative or unreported IFA level, only 15 (28.8%) were commenced on continuous B12 therapy, further showing reflex testing changed clinical management as IFA positive samples were seen to favourably be commenced on B12 therapy independent on their recorded B12 level. This suggests clinical management were dependent on IFA result. Some of the IFA tests were not reportable as B12 therapy interferes with the assay. IFA is strongly specific (98%) for pernicious anaemia in comparison to GPC antibodies (Wong, 2015). Establishment of 'normal' reference ranges of B12 and the ideal test to confirm B12 deficiency remains challenging.
Limitations included small sample size and incomplete SCR access.

Conclusion

This retrospective sampling study questions the validity and clinical utility of reflex testing of IFA in samples positive for GPC antibody.
More improved communication between the laboratory and primary care may help in development of robust guidelines on screening for pernicious anaemia and help to improve patient outcomes.

References

Wong, C. (2015). Vitamin B12 deficiency in the elderly: is it worth screening? *Hong Kong Medical Journal*, 21(2). doi:https://doi.org/10.12809/hkmj144383.

Acknowledgements

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