THE ROLE OF IGA & IGG CLASSES OF ANTI-TISSUE TRANSGLUTAMINASE IN COELIAC SCREENING

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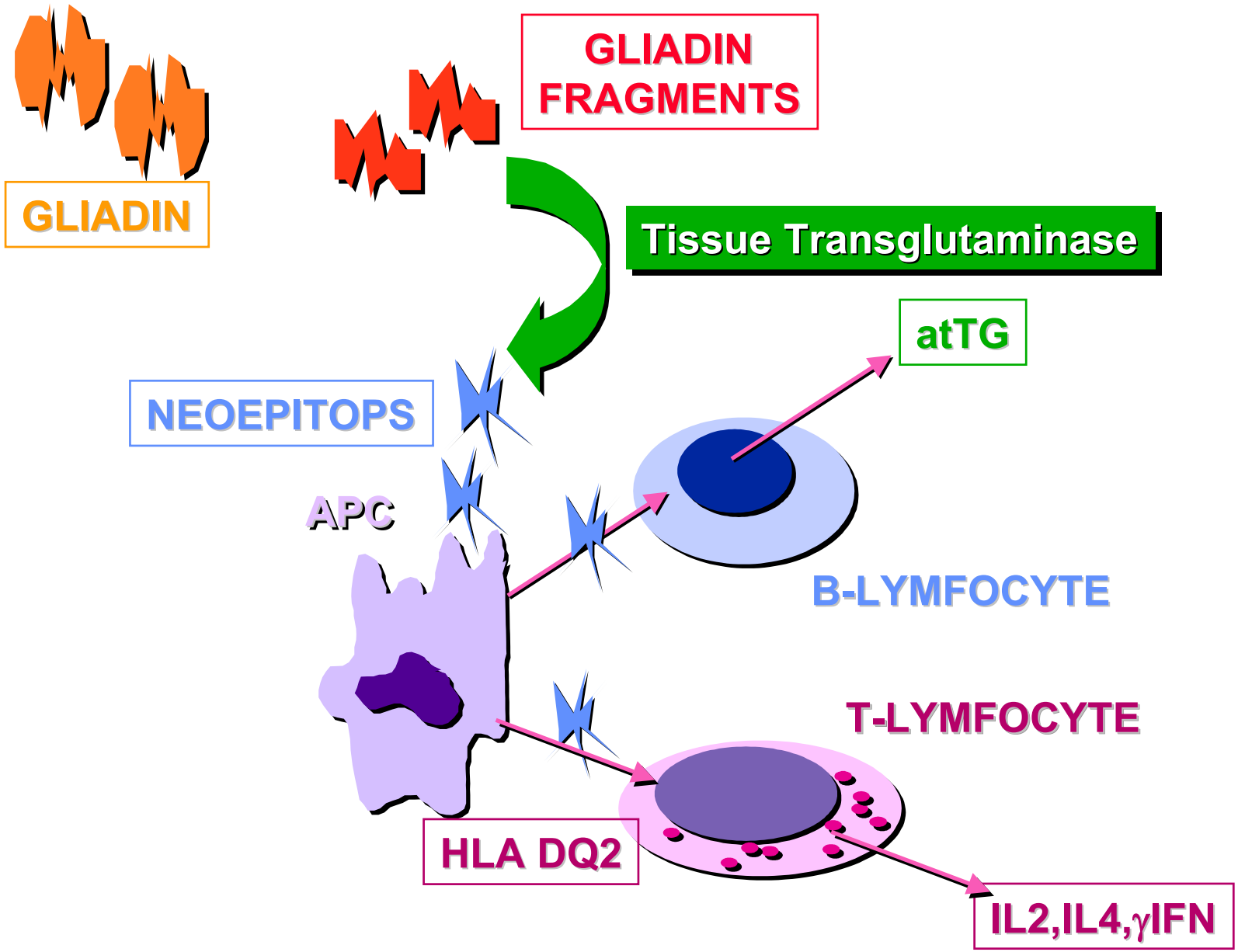
INTRODUCTION

Coeliac disease (CS) - gluten sensitive enteropathy is one of the most common underdiagnosed diseases in general practice, the prevalence of these non-diagnosed cases is 1:200 in the general population and 10-times higher (1:20) in the high-risk population with other autoimmunities as diabetes mellitus of 1st type.

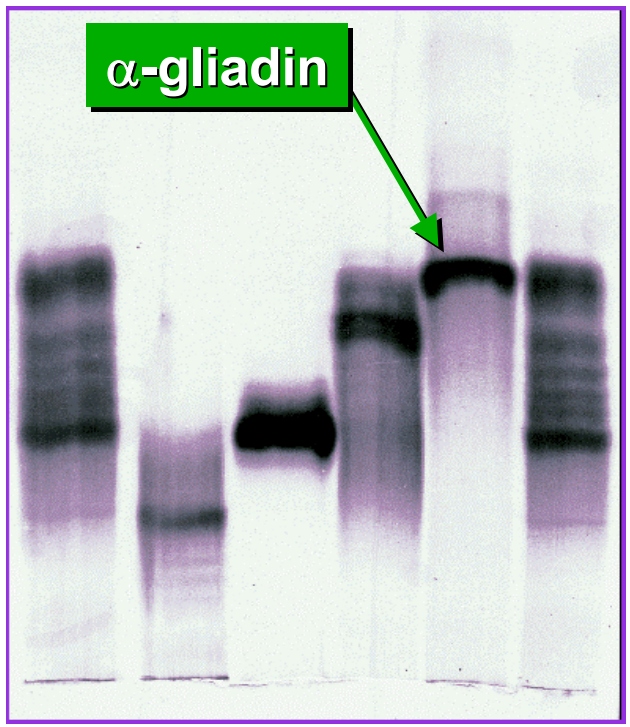
Serology markers used as a screening tool for coeliac disease are: antigliadin IgA (AGA) and IgG (AGG) antibodies, IgA antibodies to endomysium (EmA) and IgA antibodies to tissue transglutaminase (atTG), which has recently been identified as autoantigen recognised in coeliac disease.

Antibodies of IgG class should increase the clinical efficiency of serology screening, especially in cases with IgA deficiency. Our preliminary results with ELISA IgG anti-tissue transglutaminase (BioVendor, DPC-Millenium) indicate positivity of IgG-atTG in 3 of 22 patients having positive IgG-AGG antibodies but negative IgA-AGA, EmA as well as IgA-atTG.

TRANSGLUTAMINASE in CS



METHODS



Purification of gliadins by starch-gel electrohoresis in aluminium-lactate

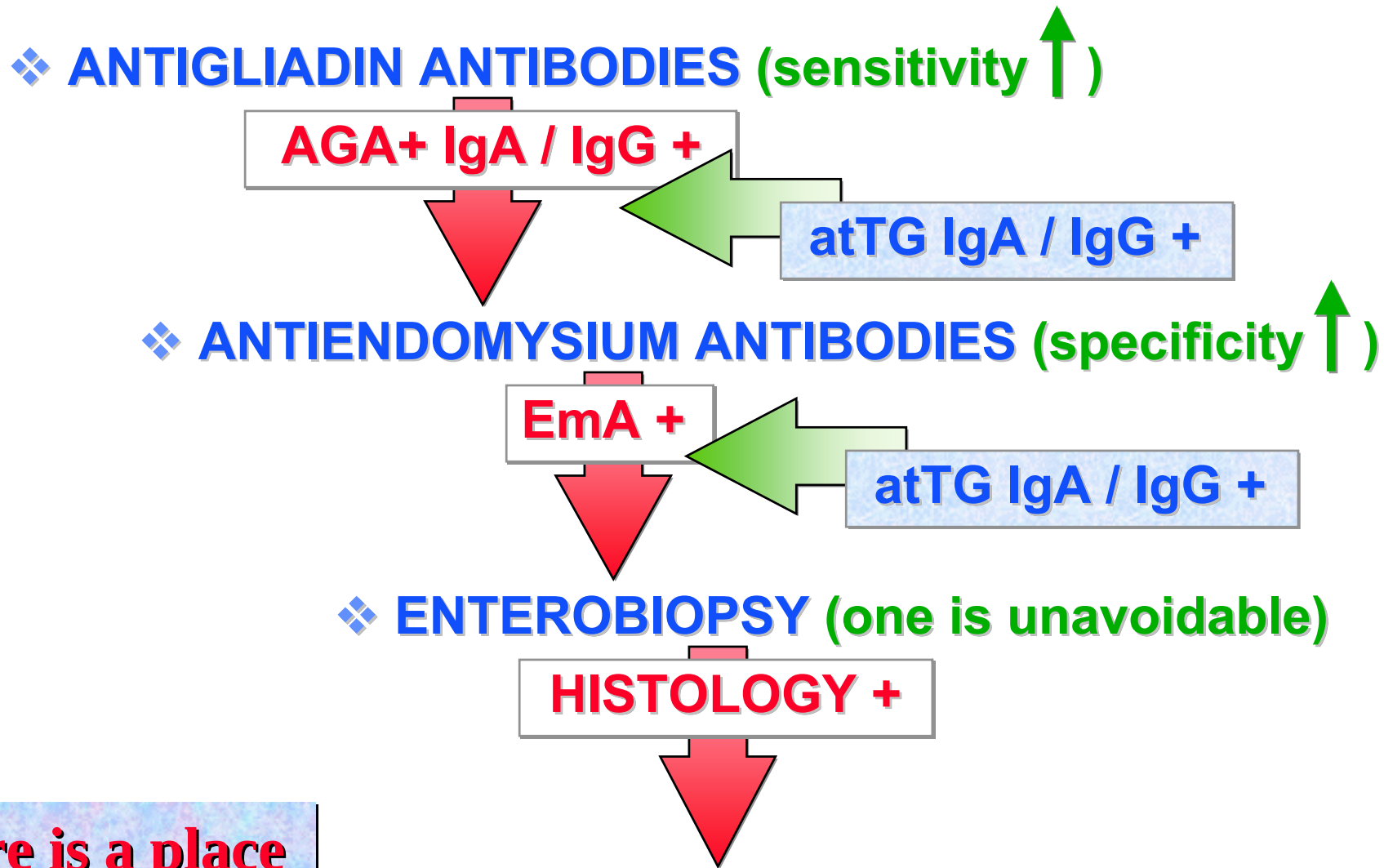
Anti-gliadin antibodies AGA of IgA and IgG, in lab. developed ELISA method with purified α-gliadin as antigen, refer.values of IgA index < 30, IgG index < 30

Anti-endomysium antibodies EmA of IgA class, immunofluorescent assay with Immco kit using primate smooth muscle slices evaluated at dilution 1:20 (positive,negative)

Anti-tissue transglutaminase atTG of IgA class, routinely used is Genesis kit with guinea pig antigen, refer.values < 10 IU/ml

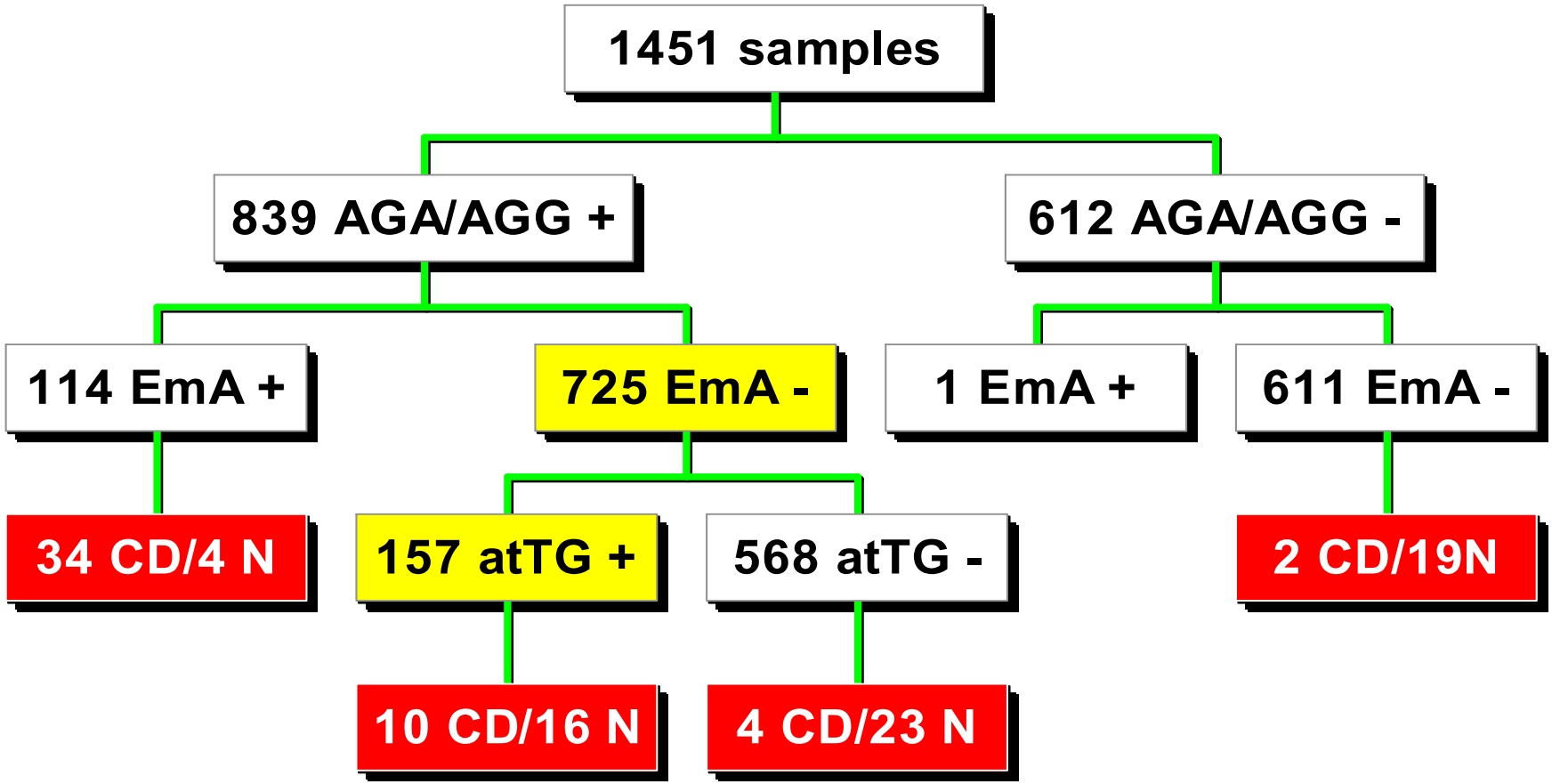
Anti-tissue transglutaminase atTG of IgA and IgG classes for this study was DPC ELISA kit with human recombinant TG as antigen, Refer.values for IgA < 10 IU/ml, IgG < 12 IU/ml

SCREENING STRATEGY



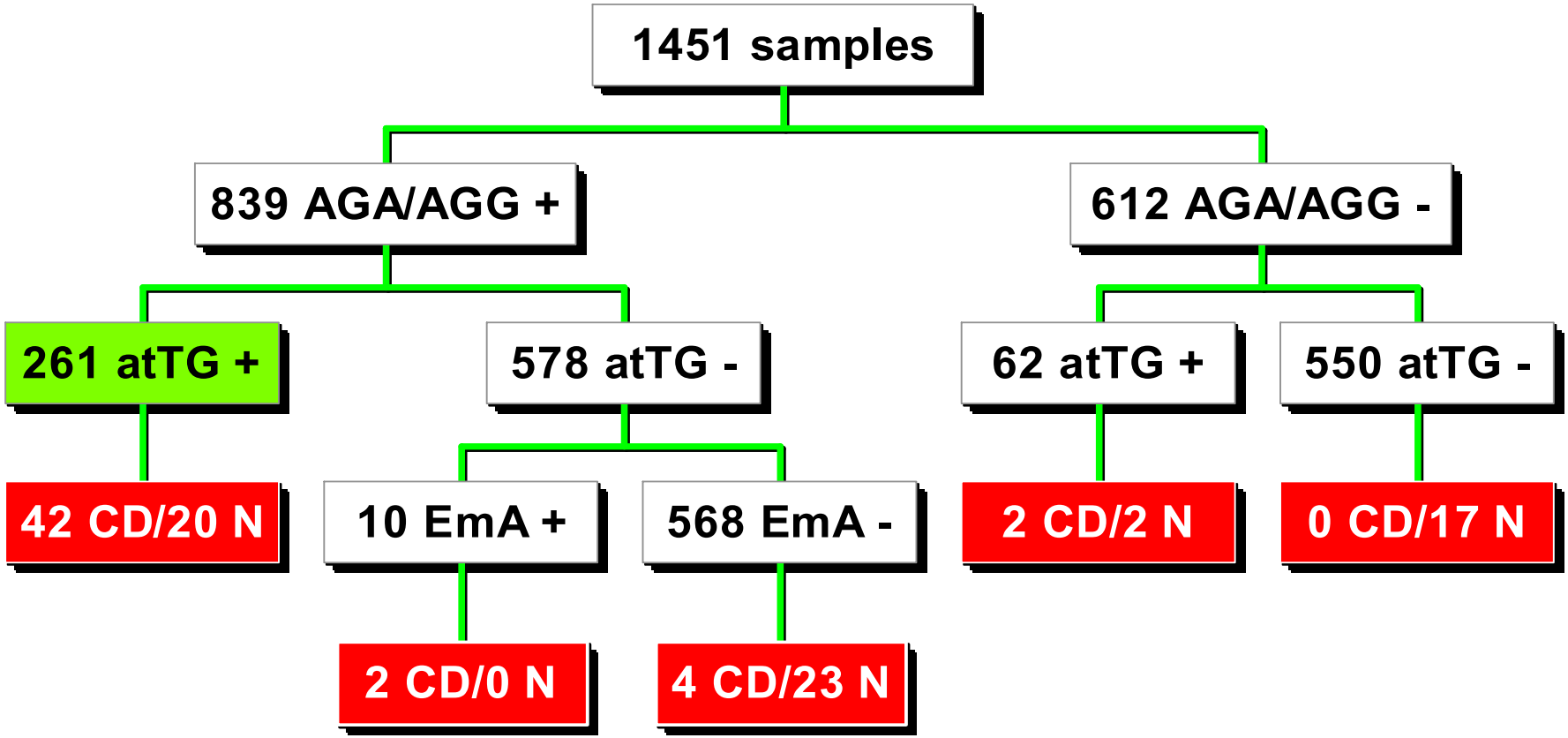
Where is a place for atTG IgG ?

ALGORITHMS - 1



Analysis of distribution of 50 cases with biopsy-confirmed CD and 62 cases with normal histology in the group of 1451 patients with all four CD-markers determined by screening algorithm with AGA/AGG as the first step followed by EmA as the second.

ALGORITHMS - 2



Analysis of distribution of 50 cases with biopsy-confirmed CD and 62 cases with normal histology in the group of 1451 patients with all four CD-markers determined by screening algorithm with AGA/AGG as the first step followed by atTG as the second.

SUMMARY of RESULTS

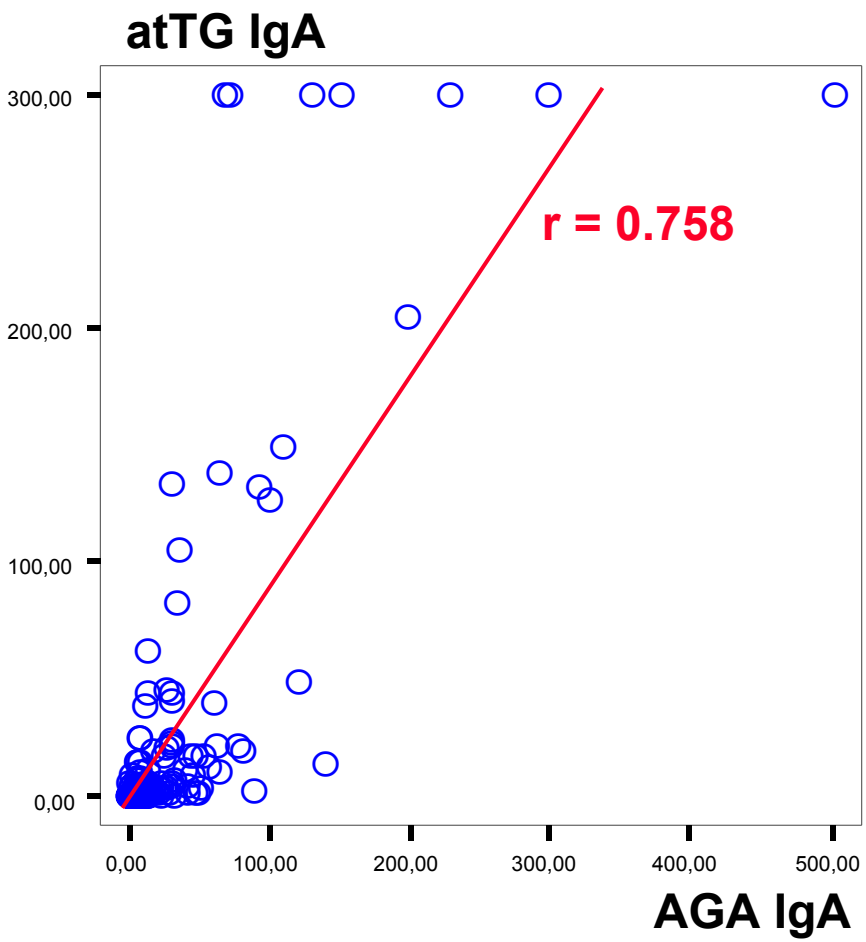
We evaluated four serology markers of coeliac disease (CD) - antigliadin IgA/IgG antibodies (AGA/AGG) with purified α-gliadin, antiendomysium IgA antibodies (EmA) and anti-tissue transglutaminase IgA (atTG) antibodies - determined in 1451 serum samples, and analysed in respect to different screening algorithms. The subgroup of 119 cases undergoing small intestinal biopsy was used to calculate sensitivity and specificity of CD markers, the highest sensitivity 94% for AGG and the highest specificity 93.5% for EmA were found. All coeliac patients were detected using the parallel combination of all four CD markers, resulting in 100% sensitivity.

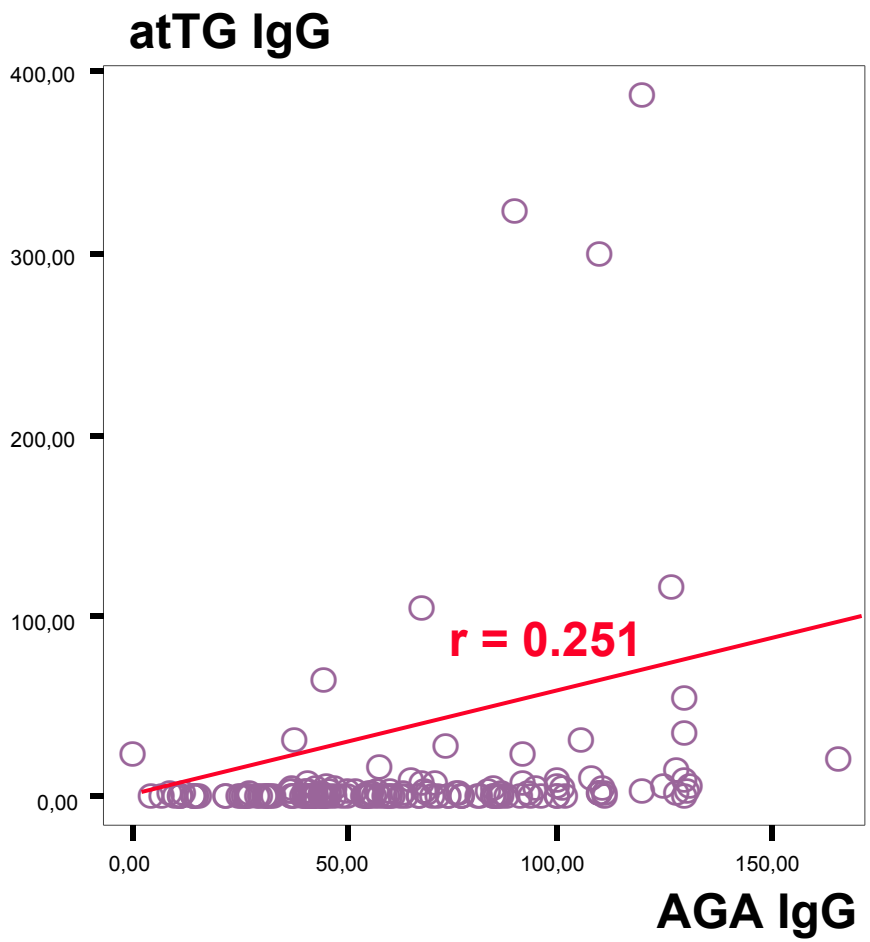
Six ELISA methods of atTG determination were compared with respect to impact of human recombinant antigen and IgG class of atTG. Antibodies to tissue transglutaminase (atTG) could be recommended as one of serology markers of CD for screening. ELISA determination of IgA atTG with human antigen could raise the specificity especially in screening of patients with other autoimmune diseases.

Autoantigens of CD and diabetes mellitus 1st type were determined in 139 diabetic patients. The atTG IgA mean value (16.7 UI/ml) was higher in GAD positive subgroup, in which at least one CD marker was positive in 83.6% subjects. In GAD negative subgroup was atTG IgA 8.73 UI/ml only and at least one CD marker was positive in 57.4% subjects.

CORRELATIONS - 1

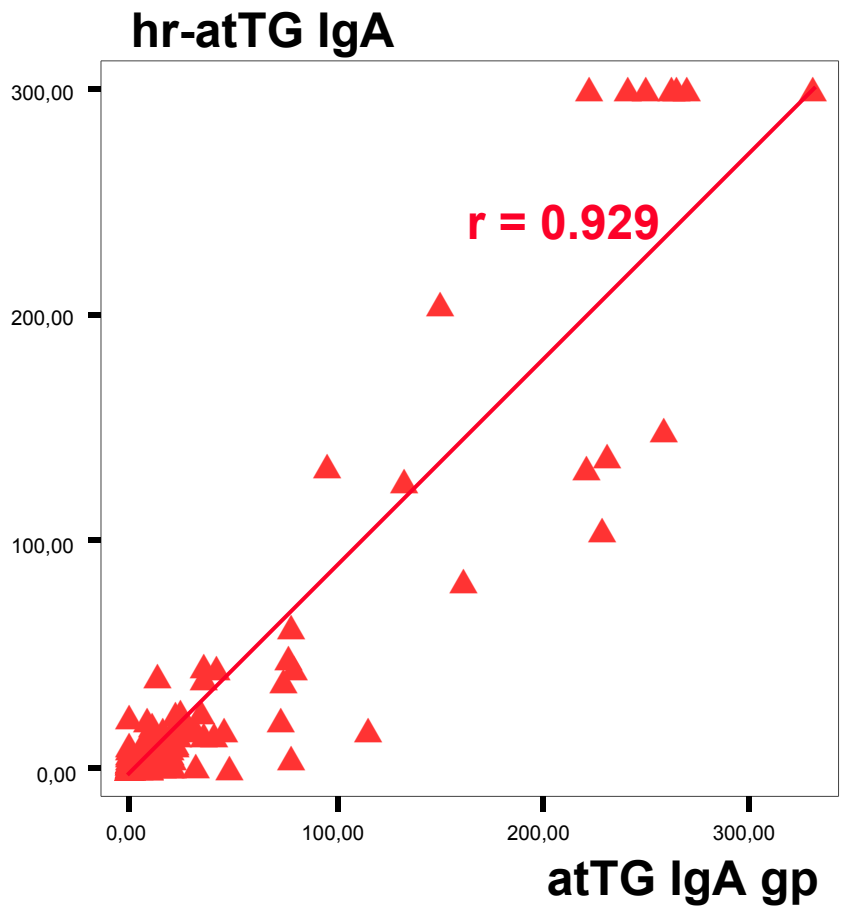
Correlations of anti-tissue transglutaminase (DPC) and anti-gliadin antibodies in IgA class (r=0.758) and IgG class (r=0.251), both statistically significant on p < 0.01

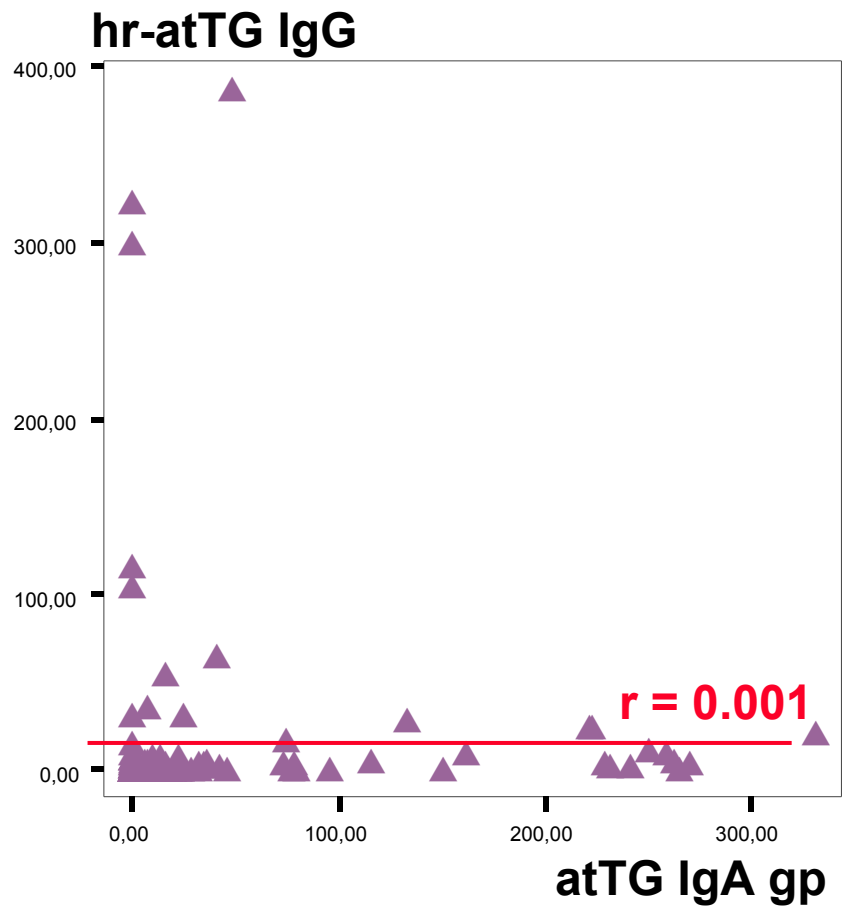




CORRELATIONS - 2

Correlation of new human recombinant anti-tissue transglutaminase DPC with guinea-pig Genesis in IgA class is highly significant (r=0.929) on p < 0.001 and no correlation was found between hr-atTG of IgG class and Genesis IgA.





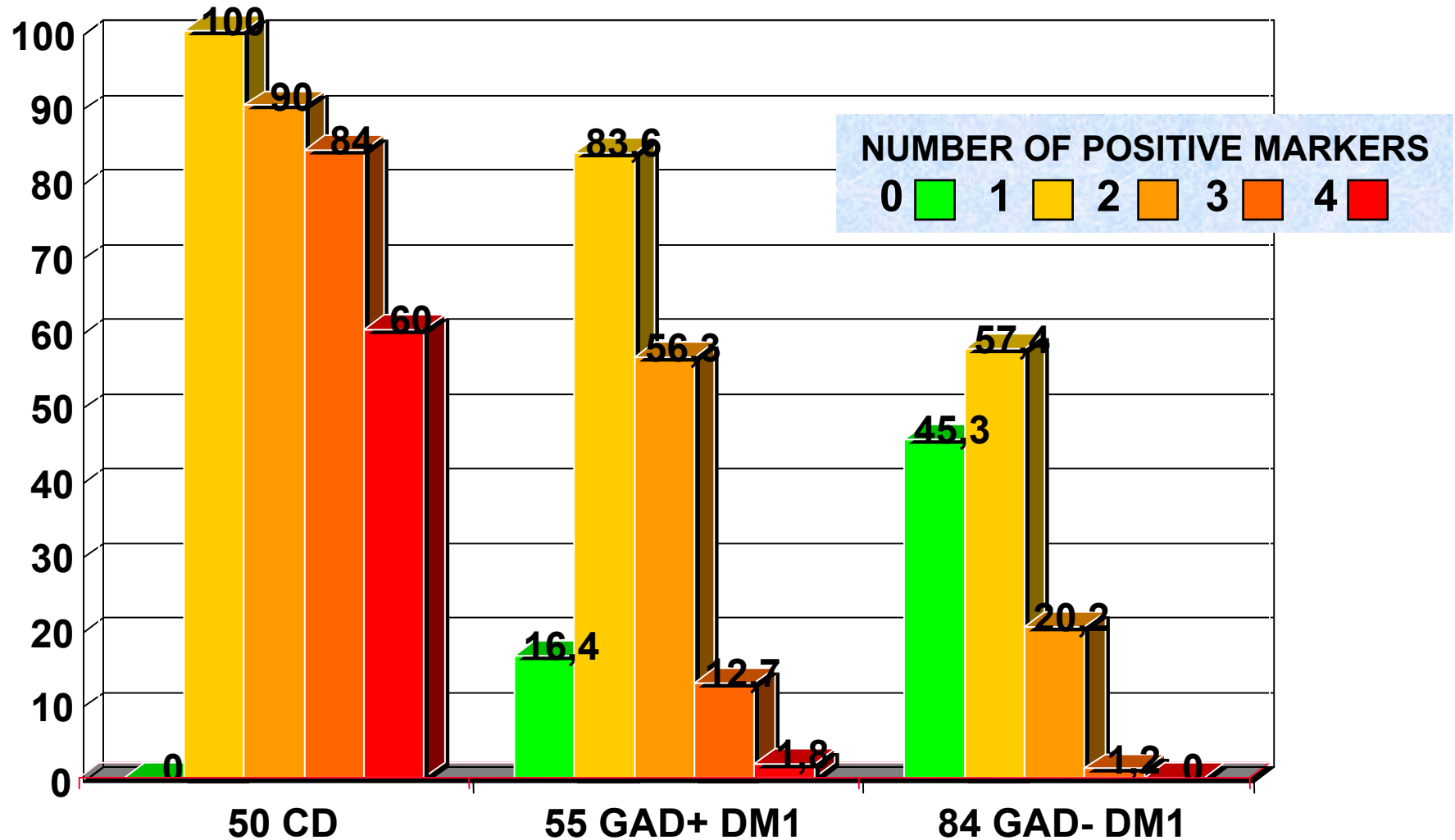
ELISA for atTG IgA

Several studies had been performed to compare various ELISA assay kits for anti-tissue transglutaminase, different in antigen, Ca^{2+} activation and with different reference values. All these methods were compared to ELISA used routinely in our laboratory - Genesis IgA with guinea pig Ca^{2+} activated antigen.

Concordance of positivity/negativity with EmA antibodies was tested using Cohen's kappa coefficient of agreement (number of samples).

ELISA KIT	ANTIGEN / ACTIVATION	VALUE	GREY ZONE	CORR.	COHEN's κ
DPC	HUMAN RECOM.	> 10	7 - 10	0.93	0.61 (153)
IMMCO	GUINEA PIG / Ca+	> 25	20 - 25	0.79	0.45 (161)
MEDIPAN	GUINEA PIG / Ca+	> 25		0.59	0.26 (40)
ORGENTEC	HUMAN CELLS / Ca-	> 15		0.92	0.31 (119)
GENESIS	GUINEA PIG / Ca+	> 10		n.d.	0.40 (1450)
KRČ - AVČR	GUINEA PIG / Ca+	> 25		0.81	0.57 (40)

CD MARKERS & IDDM



Cummulative percentage of CD-markers (zero, at least one, any two, any three or all four) positive in three groups of patients: florid coeliac disease (50 cases), diabetes mellitus GAD-positive (55 cases) and diabetes mellitus GAD-negative (84 cases).

CONCLUSIONS

In this study we compared different algorithms using four serology markers of CD in a group of 1451 patients. The correlation of two ELISA methods (Genesis, DPC Millenia) of atTG IgA class was high (r = 0.930), correlations of atTG (DPC) and antigliadin (AG) antibodies were significantly higher in the IgA class than IgG class, Spearman's coeff. were 0.607 and 0.423 respectively.

The clinical benefit of IgG atTG antibodies determination could be exhibited by 7 cases highly positive in IgG class but negative in IgA class of atTG, or by 5 cases in the screening strategy positive with IgG atTG from 78 cases negative in all IgA class antibodies (AGA, EmA, atTG). ELISA determination of IgA atTG with human antigen could raise the specificity especially in screening of patients with other autoimmune diseases.

ELISA determination of IgG class of atTG could be recommended as additional serology marker of silent coeliac disease especially in IgA deficiency and other cases with negative EmA or IgA-atTG antibodies.

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