

Comparison of methods for determining antibodies against *Borrelia* spp. in early and late forms of Lyme disease

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Main goals

- to compare TestLine's Clinical Diagnostics serological screening kits for the CLIA and ELISA methods in groups of patients with early and late forms of Lyme borreliosis (LB) and healthy controls, using Microblot-Array (MBA) for confirmation
- to determine differences in antigen reactivity in groups of patients with early and late LB and healthy controls
- to determine changes in antibody formation and differences in antigen reactivity in patients with early LB before treatment and one month after treatment

Introduction

- Lyme borreliosis (LB), the most common vector-borne disease in Europe, is caused by *Borrelia burgdorferi* sensu lato and transmitted via tick *Ixodes ricinus*. The Czech Republic is an endemic country for LB, on average, 4000 cases of LB are reported annually, and the incidence is around 40 cases per 100,000 population.
- The most frequent symptom was erythema migrans (EM), reported on average in 67% LB patients (around 3000 cases per year).
- Lyme neuroborreliosis (NB) was recorded on average in 13% (around 550 cases per year).

Results

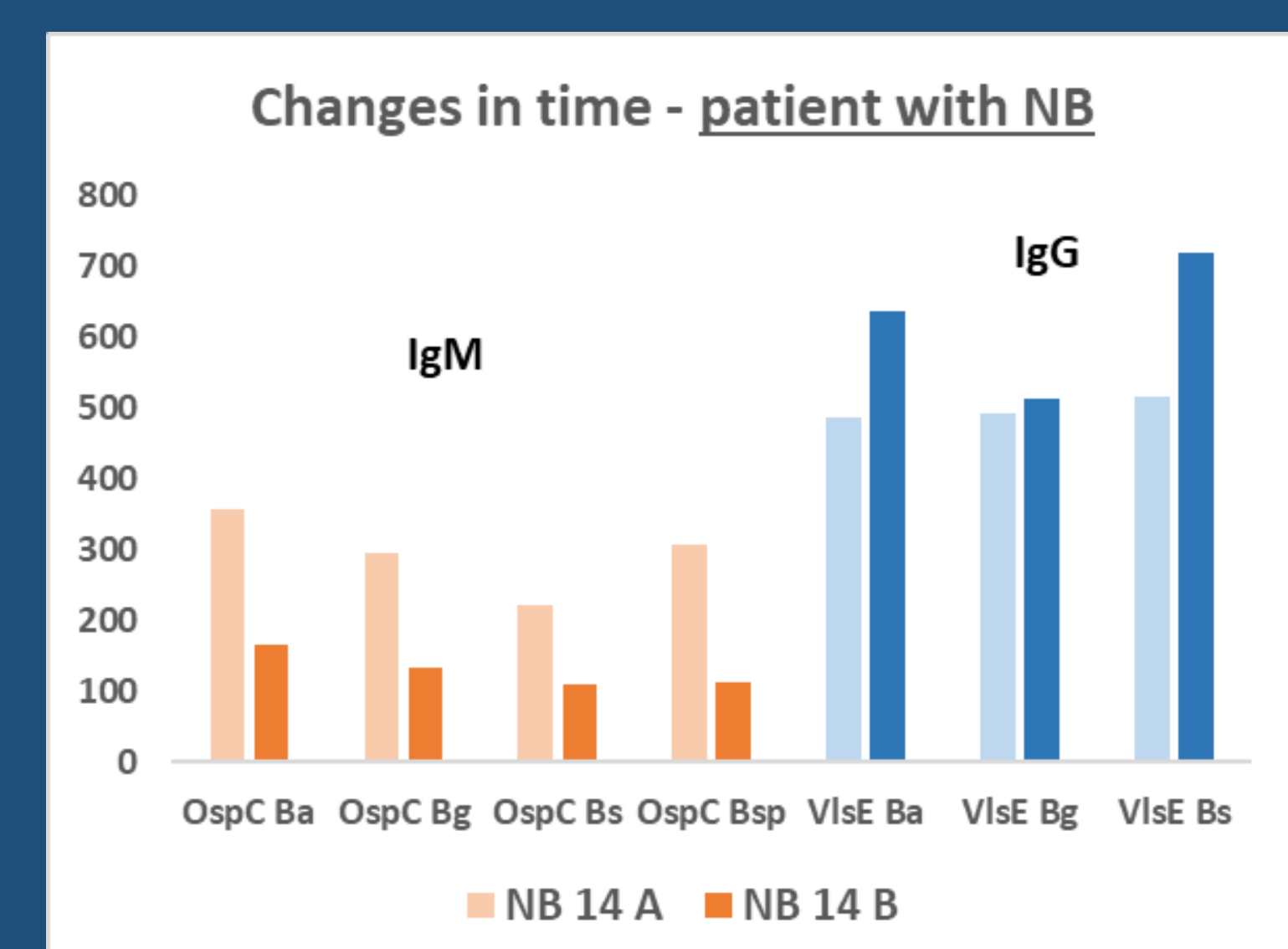
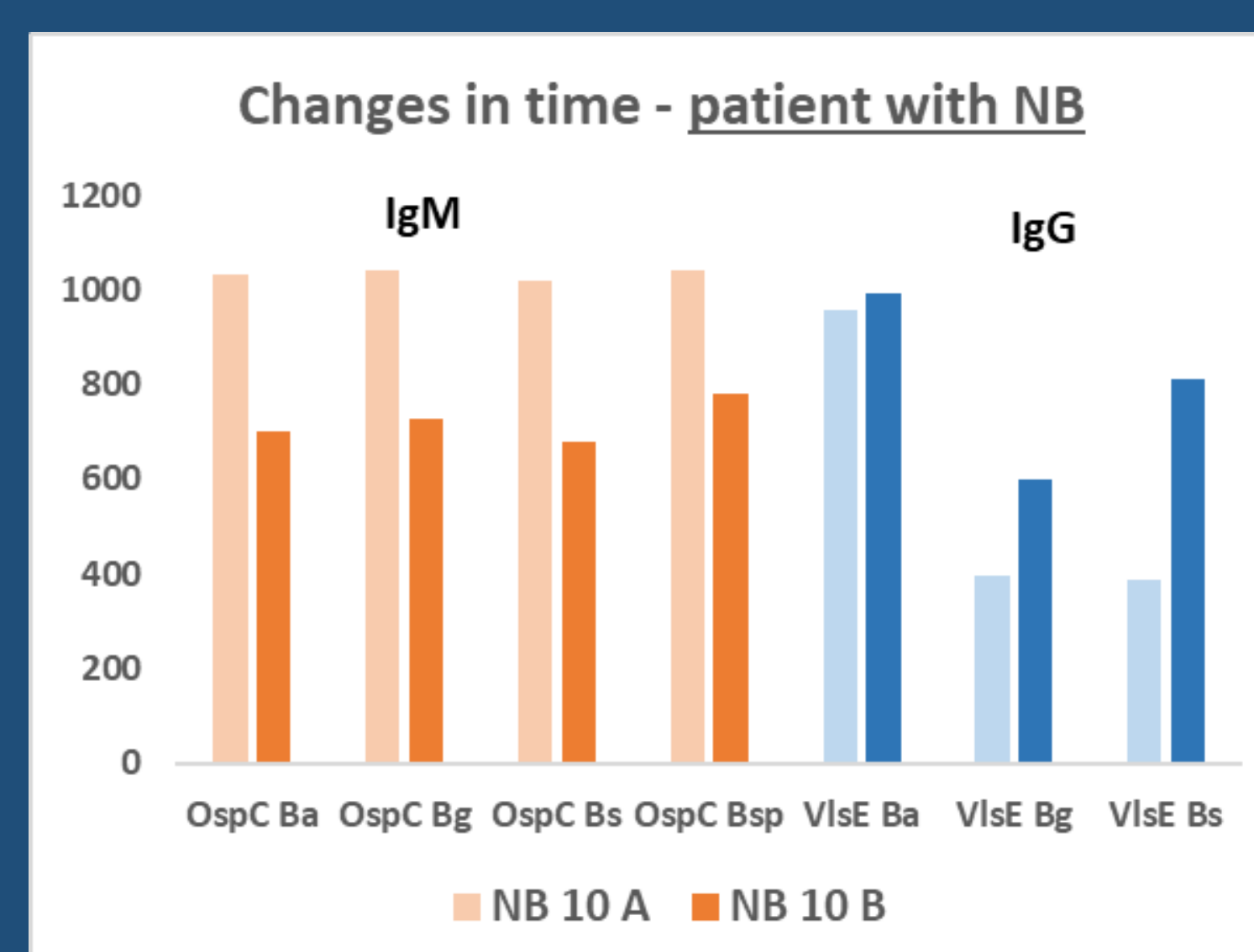
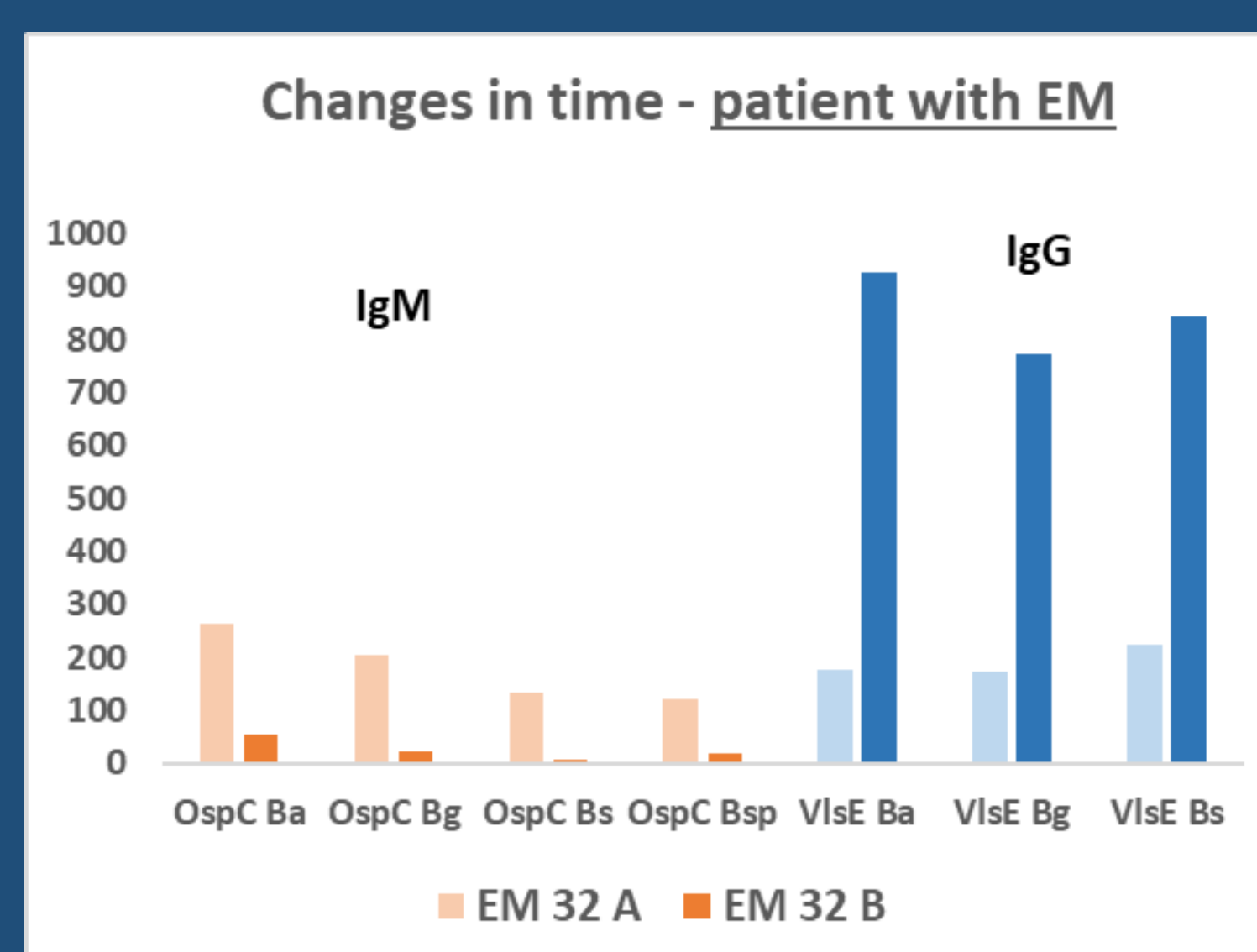
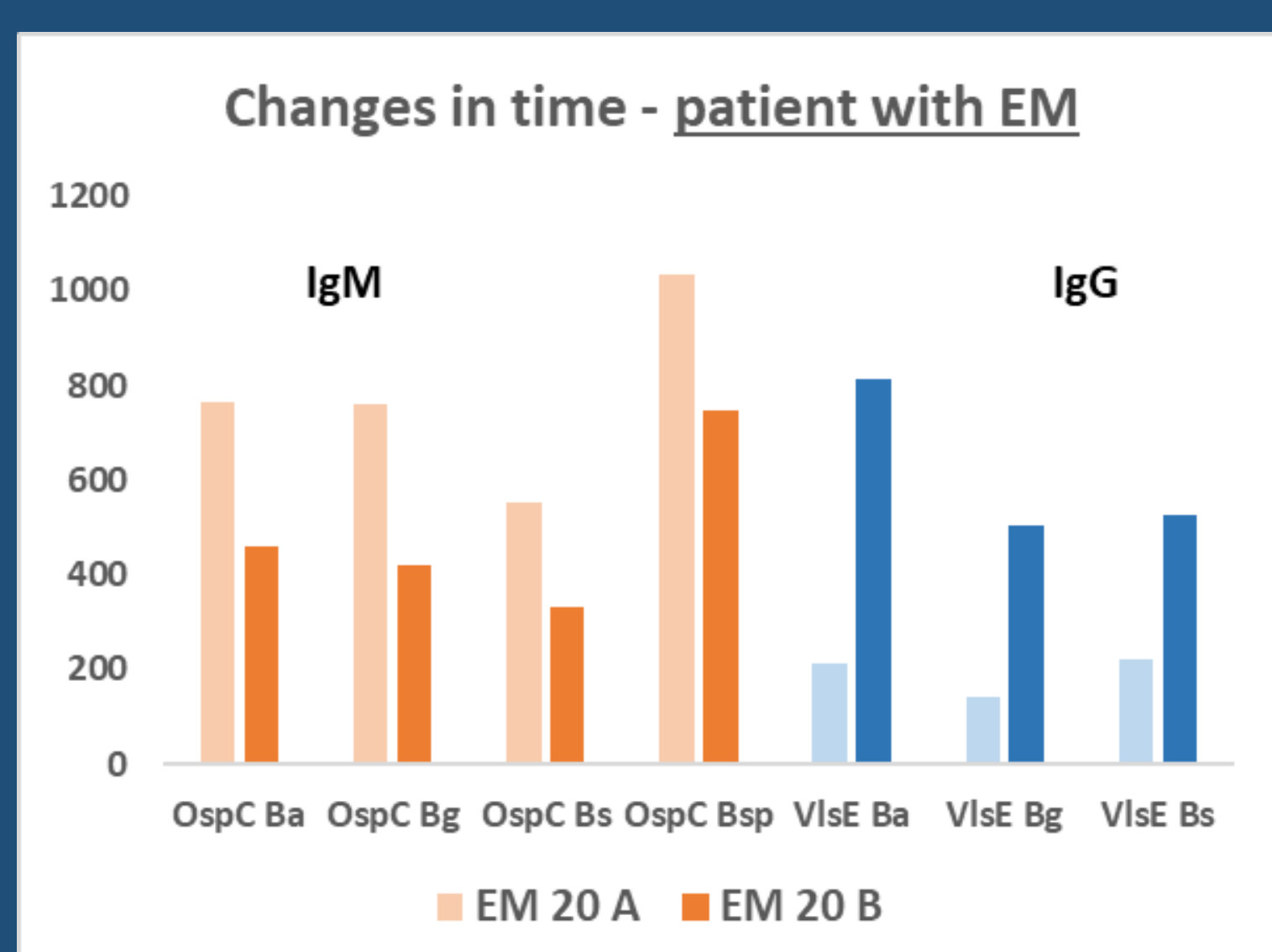
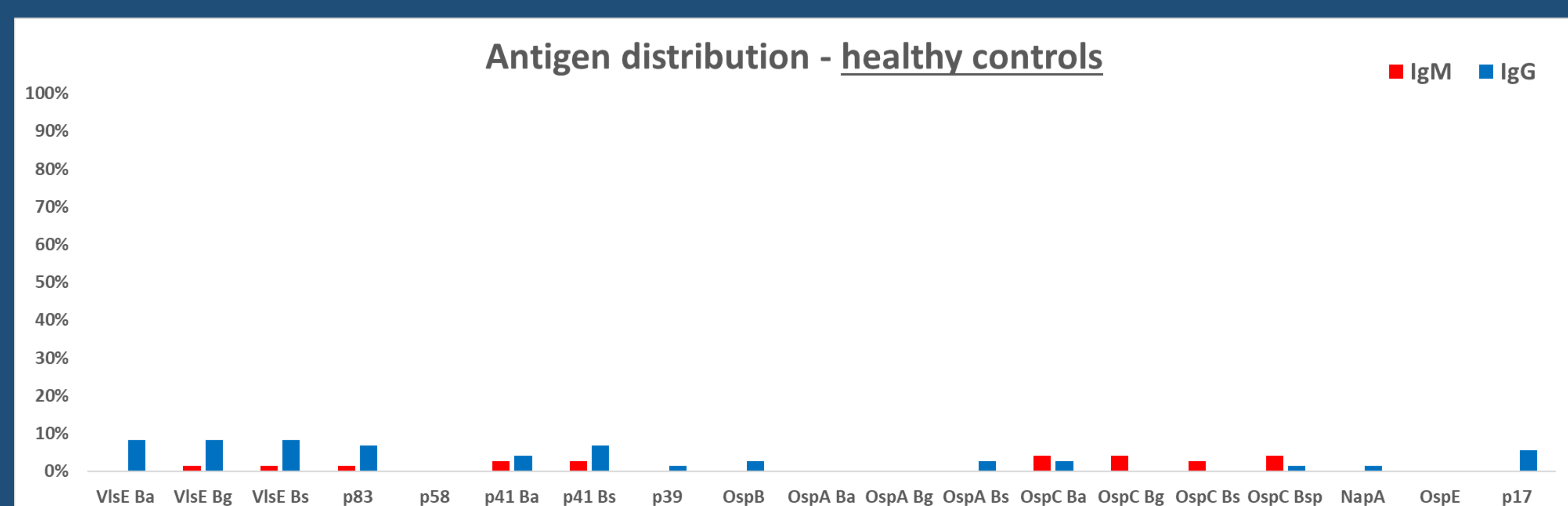
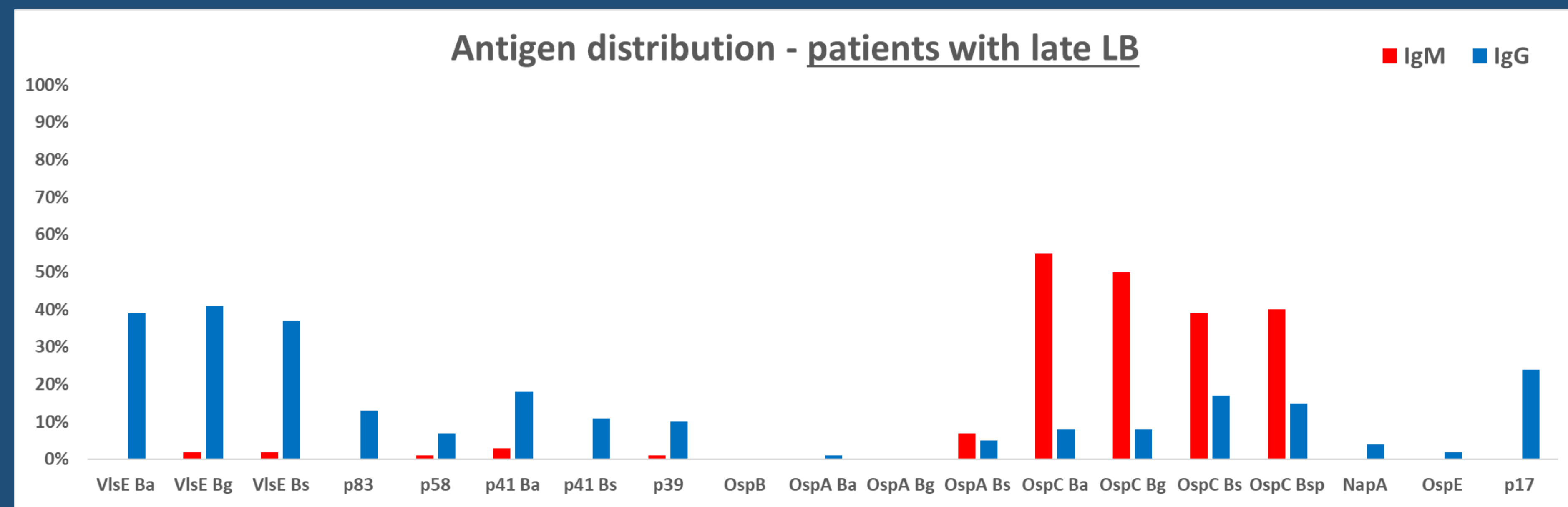
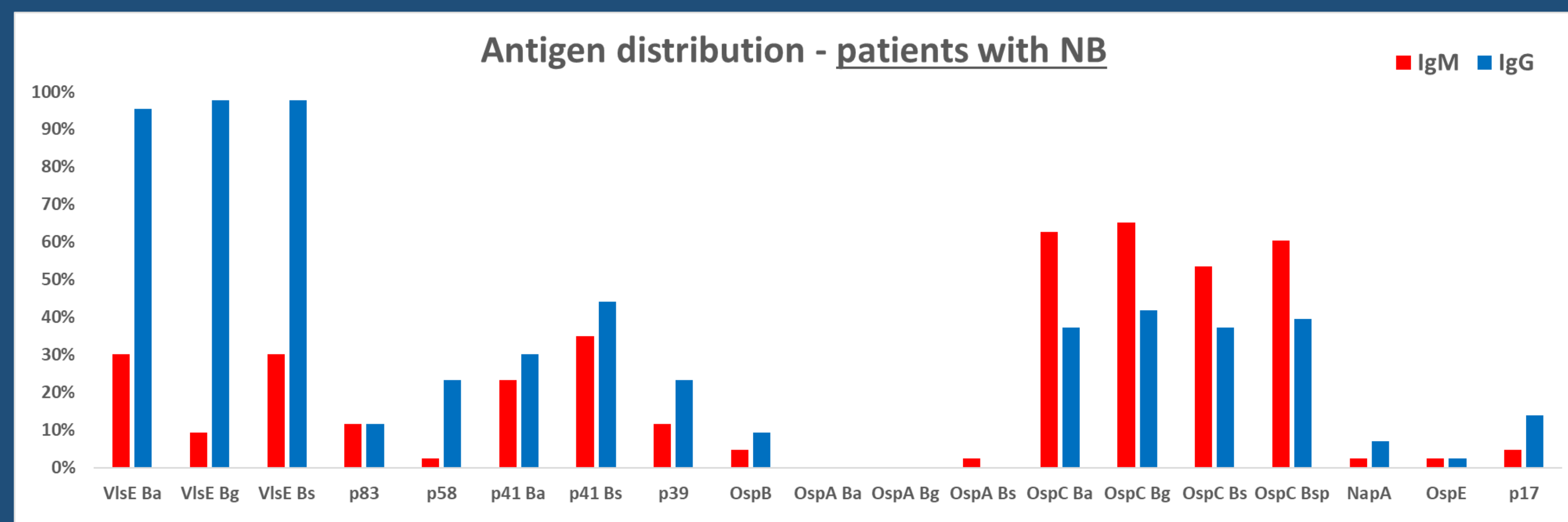
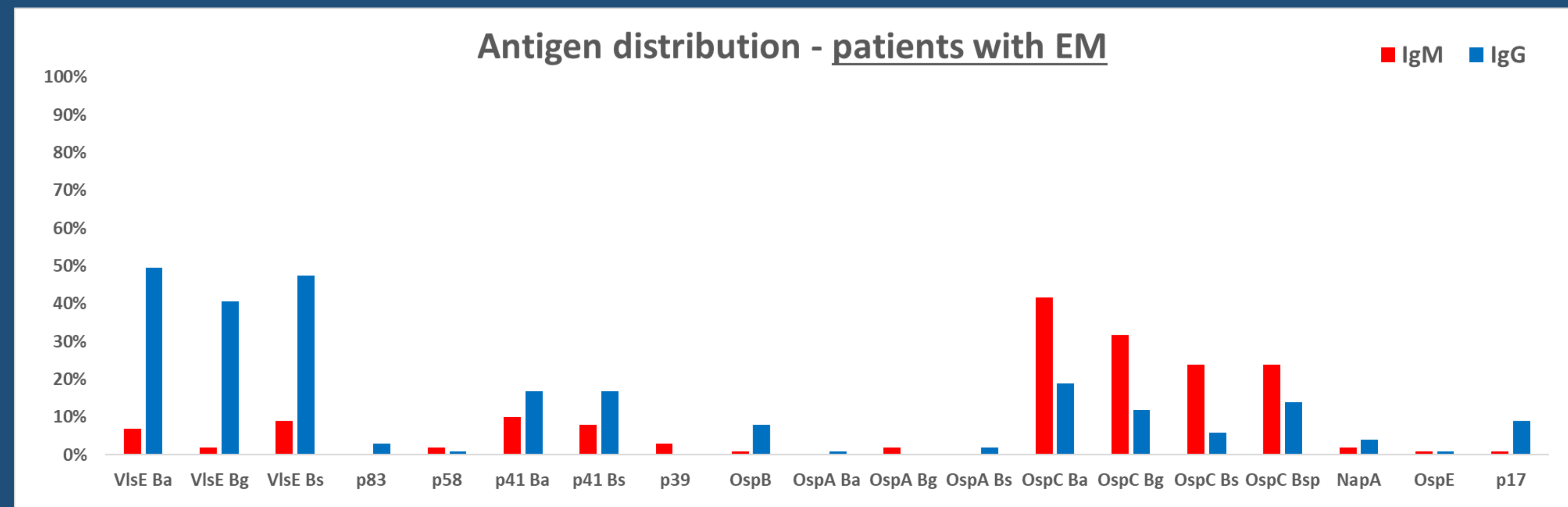
- to compare serological screening methods:**
 - a high agreement between both CLIA and ELISA screening methods: **97.4% in IgM** and **93.3% in IgG**
 - using MBA as a reference method: the **sensitivity of CLIA was 92.4% (IgM) and 84.1% (IgG)** and **specificity 89.1% (IgM) and 91.7% (IgG)**
- the most **frequently reacting IgM antigens**
 - in **early LB (EM and NB): OspC, p41, p39** and **VlsE**
 - in **late LB: OspC** and **OspA**
 - in **healthy controls:** IgM antigens react only exceptionally
- the most **frequently reacting IgG antigens**
 - in **early LB (EM and NB): VlsE, p41, OspB, OspC**
 - in **NB additionally: p39** and **p58**
 - in **late LB: VlsE, OspC, p41, p17, p39, p83, p58**
 - in **healthy controls: VlsE, p83, p41, p17**
- the **changes in time in early LB patients:**
 - the changes of IgM antibodies in EM patients were **more pronounced in the MBA** compared to CLIA
 - after one month: IgG antibodies increased** (VlsE, OspC) in patients with **EM and NB**
 - after one month: IgM antibodies decreased** (OspC, p41) in patients with **NB**

Methods

- sera from patients with the early form of LB were used:
 - 101 patients with erythema migrans (EM)**
 - 43 patients with neuroborreliosis (NB)**
- 100 sera from patients with the late form of LB**
- 73 sera from healthy controls**
- for 43 patients with EM and 13 patients with NB
 - two samples: before treatment and one month after
- TestLine Clinical Diagnostics kits were compared:
 - ELISA Borrelia recombinant IgM and IgG**
 - CLIA Borrelia recombinant IgM and IgG**
 - MBA Borrelia IgM and IgG**

relative agreement IgM		CLIA		
		positive	borderline	negative
ELISA	positive	91,8%	5,7%	2,5%
	borderline	46,7%	46,7%	6,7%
	negative	2,3%	9,8%	87,9%

relative agreement IgG		CLIA		
		positive	borderline	negative
ELISA	positive	86,2%	2,3%	11,5%
	borderline	11,1%	27,8%	61,1%
	negative	0,0%	0,0%	100,0%



Literature

Orlkov, H., Kybicov, K., Mal, M. et al. Surveillance and epidemiology of Lyme borreliosis in the Czech Republic in 2018 and 2019. *Biologia* 77, 1651–1660 (2022).
 Krbkov L., Kybicov K., Pcha D., Rohčov H., Smřkov D. [Guideline for the diagnosis and treatment of Lyme borreliosis]. *Klin Mikrobiol Infekc Lek.* 2018 Sep;24(3):88-99. Czech. PMID: 30747990.

Acknowledgement

This study was supported by MH CZ - AZV, NU23-05-00511.

Conclusion

- The comparison shows that the new generation of CLIA serological methods has the same sensitivity and specificity as ELISA, and the results also agree with the latest MBA confirmation method.
- The results of the development of antibodies in various manifestations of LB can be helpful in the diagnosis of the disease and the subsequent treatment of the patient.