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When a *Neisseria meningitidis* PCR limitation contributes to an immunological disease diagnosis

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Capsule;
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Dear editor,

Capsulated *Neisseria meningitidis* is responsible for invasive meningococcal disease (IMD). Only few non-capsulated IMD are described in the literature.[1,2]

A 36-year-old patient, without previous medical history, presented for stiffness and headache. He had fever (39 °C), photophobia, vomiting, purpura on ankles and torso. Blood leukocyte count was 29,98 × 10^9 cells/L (95.5 % polymorphonuclear), C-reactive protein 33.77 mg/dL, procalcitonin 18.4 ng/mL. He was given 2 g cefotaxime and 20 mg dexamethasone few hours before lumbar puncture and blood sample. Evolution was quickly favorable.

The cerebrospinal fluid revealed a leukocyte count of 20,50 × 10^9 cells/L (92 % neutrophils), protein 729 mg/dL, glucose <4 mg/dL, lactic acid >16 mmol/L, and gram-negative diplococci were observed on the Gram stain. Blood and cerebrospinal fluid remained sterile. FilmArray® meningitis/encephalitis panel multiplex PCR (BioMérieux, Marcy l'Étoile, France) and *N. meningitidis* real-time PCR were negative. By 16S rRNA sequencing, a 677 bp DNA bacterial fragment was obtained and was 99.5 % identity with *Neisseria* sp. strains including *N. meningitidis*. The French National Reference Laboratory detected a non-capsulated *N. meningitidis* strain with two routinely used PCR: the *sodC* (Cu–Zn superoxide dismutase, a generic conserved gene in *Neisseria meningitidis*) PCR was positive while the *ctrA* (capsule transport gene) PCR was negative, suggesting a capsule null locus (cnl) strain.[3] The strain belonged to the clonal complex CC175 (Table 1). This finding led us to look for other factors contributing to IMD such as underlying disease: only hypocomplementemia was detected (complement hemolytic 50 level <14 U/mL).

The major virulence factor leading to IMD is the polysaccharide capsule. Non-capsulated *N. meningitidis* strains can colonize nasopharynx of healthy subjects and are low-pathogenic strains, because not resistant to complement mediated lysis and opsonophagocytosis.[3,6] Few infection cases are reported in the literature, mostly in immunocompromised patients. In 2018, Kurose summarized 13 cases, including nine meningitis: three patients with C6 deficiency, one patient with IgG4-related disease, and five with unknown risk factor.[6]

Patients with a deficiency in the terminal complex and the factor properdin of the complement system have an increased risk of recurrent capsulated IMD. Less frequently, IMD caused by non-capsulated strains have been reported.[7] Rosain reported 61 IMD among subjects with terminal complement pathway deficiencies: 8 % were due to non-capsulated strains.[2] Ladhani described 20 IMD among patients with inherited and acquired complement deficiency: four among nine IMD in patients on eculizumab therapy were due to non-groupable or group E (less virulent) isolates.[1] Thus, it is necessary to investigate immune comorbidities after non-capsulated IMD.

The capsule being a virulence factor responsible for IMD, *ctrA* is frequently used to detect *N. meningitidis*, including in commercial PCR assays. Nevertheless, it has always been described that nucleotide substitution/rearrangement in *ctrA* gene may be responsible for false-negative results, including in invasive strains.[4] Carriage isolates may even lack this gene.[5] Then our last taking-home message is that

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Table 1 Molecular biology tests performed on the cerebrospinal fluid sample.

Tests performed	Targets	Result
BioFire® FilmArray meningitis/encephalitis panel (BioMérieux, Marcy l'Étoile, France)	« Encapsulated *N. meningitis* (groups A, B, C, W, Y) and DNA from a strain with a variant *ctrA* gene »	Not detected
N. meningitidis real-time PCR (Diagenode Diagnostics, Liège, Belgium)	*ctrA* (capsule transport in encapsulated strains) *csaB* (group A) *csb* (group B) *csc* (group C) *csw* (group W) *csy* (group Y)	Not detected
16S rRNA sequencing (377 ABI Prism; PE Applied Biosystems, Foster City, CA., USA) Analysis with BLAST system	Universal 16S RNA PCR	*Neisseria* sp.
sodC and *ctrA* real-time PCR (Centre National de Référence des méningocoques, Institut Pasteur, Paris, France)	*sodC* (Cu—Zn superoxide dismutase gene) *ctrA* (capsule transport in encapsulated strains)	Detected Not detected
MLST (Centre National de Référence des méningocoques, Institut Pasteur, Paris, France)	Multilocus sequence typing	Clonal complex CC175
Final result		Non-capsulated *N. meningitidis*

biological tests cannot be 100 % sensitive. This warrants a systematic *sodC* PCR in addition to *ctrA* PCR since *sodC* is conserved in *N. meningitidis* strains regardless of capsule status but not in other *Neisseria* species.[5] Presume meningococcal disease with negative PCR may be due to a cnl strain and clinical history remains the best indicator for diagnosis.

Declaration of competing interest

None.

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