Sir,

We appreciate the comments made by Raymond et al.\(^3\) on our recent study,\(^2\) in which we evaluated the accuracy of several genotypic predictors of HIV tropism, taking as reference the results obtained using a phenotypic assay (Phenoscript\(^6\)) in 150 plasma samples collected from HIV-1 patients infected with either B (n=75) or non-B clades (n=75). One of the genotypic approaches evaluated was Delobel’s rule, which refers to the interpretation algorithm proposed by Delobel et al.\(^3\) a few years ago. At that time Delobel and colleagues reported an improved genotype–phenotype correlation for predicting HIV tropism by combining the 11/25 and net charge rules. Delobel et al.\(^3\) said ‘the presence of CXCR4–using viruses in HIV-1 quasi-species was suspected when an R or K amino acid at V3 position 11 or 25 was detected in major or minor species on the electropherogram, concomitant with a maximum net charge of ≥+5 (when considering the combination of codons that resulted in the highest net charge)’. Following that description, we did our analyses. Therefore there is no mistake in our interpretation, as samples were labelled as X4 if a basic amino acid (R or K) was recognized at positions 11 or 25 within the V3 region and if the global net charge of the V3 sequence was ≥+5.

In a subsequent publication, Raymond et al.\(^4\) suggested that prediction of CXCR4–coreceptor usage should be made considering ‘either 11R/K or 25K or both; 25R and a net charge of at least +5; or a net charge of at least +6’. We have re-analysed the 150 samples of our study using this new interpretation in order to evaluate its accuracy to predict HIV tropism using Phenoscript\(^6\) results for comparison. Overall, we found a sensitivity/specificity of 78%/86% for recognition of X4 variants in clade B viruses, which is lower in terms of sensitivity to that found using Garrido’s rule (94%/74%).

The sensitivity/specificity for detecting X4 variants in antiretroviral (ARV)-experienced patients was 75%/85%, but 40%/93% in ARV-naïve individuals. The last figures suggest a lower sensitivity than in the original report by Raymond et al.\(^5\) (63%/97%). The difference could be in part due to the variability between the two different phenotypic methods used to determine HIV tropism in both studies.

The overall sensitivity/specificity to predict X4 variants in non-B subtypes using Raymond’s rule\(^4\) was 42%/92%. These results are not in line with prior data in a different set of non-B subtypes tested by these authors that included A1, F1, G, J, CRF_01, CRF_02 and CRF_06. They found the same genotype–phenotype concordance among B (76/84) and non-B viruses (13/14). However, it is worth noting the limited size of the population examined and the difficulty of getting conclusive results.

In our dataset we have performed a specific analysis of 21 CRF02_AG specimens and found sensitivity/specificity rates of 40%/94%, while Raymond et al.\(^5\) reported figures of 70%/98% for CRF02_AG (n=52). The different sensitivities between studies are most likely due to the limited size of the populations examined and require further attention. It must be highlighted that misclassification of X4 variants may be critical in terms of clinical consequences.

In our dataset we did not have enough clade C specimens (only eight) and therefore could not derive any conclusion. We agree that specific genotypic predictors are needed for determining HIV tropism in patients infected with clade C.\(^6\) Overall, all this information highlights that further studies testing larger cohorts of patients infected with distinct non-B subtypes are required in order to establish the reliability of genotypic tools for assessing HIV tropism in clinical settings and guide the therapeutic use of CCR5 antagonists.

### Transparency declarations

None to declare.

### References

Letters to the Editor

Comment on: Cerebrospinal fluid impairs antimicrobial activity of fosfomycin in vitro

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Keywords: CSF, glucose-6-phosphate, in vitro simulation, nosocomial meningitis

Sir,

Sauermann et al.,1 recently investigated the influence of human cerebrospinal fluid (CSF) on the antimicrobial activity of fosfomycin in vitro. Bacterial time–kill curves were performed in different media using fosfomycin concentrations below and above the MIC for two Staphylococcus aureus isolates with high and low susceptibility according to BSAC and CLSI definitions. In addition, they exposed the test strains to the fosfomycin pharmacokinetic profile measured at steady-state in the CSF of a selected patient with external ventricular drain-associated infection from a study published previously by our group in CSF obtained from pooled samples.2 Despite initial killing, the in vitro simulation of the selected pharmacokinetic profile showed terminal regrowth of the S. aureus isolates. The authors deduce from their experiments that the antibacterial activity of fosfomycin is notably reduced in human CSF and may not be sufficient to achieve bacterial effects in the CNS.1 This is in contrast to the results of a previous in vivo pharmacokinetic study by our group, indicating that intravenous fosfomycin should provide sufficient antimicrobial concentrations in the CSF and, therefore, might qualify for the treatment of nosocomial intracranial infections.2 Though we cannot definitely rule out that in our study the combination of fosfomycin with other antibiotics as recommended by experimental and clinical data contributed to enhanced bacterial killing by fosfomycin with complete resolution of intracranial infection in all our patients, concerns arise when extrapolating the presented in vitro data to real-life clinical conditions.1 Unfortunately, Sauermann et al.1 do not give further information on the biochemical and cellular composition of the used CSF samples or the clinical condition of the respective patients from whom the CSF was obtained. This would have been of paramount interest for the correct interpretation of their in vitro results. It has to be emphasized that critical illness not only grossly alters the pharmacokinetics, but also influences the pharmacodynamics of anti-infective agents by a significant amount. In the case of nosocomial intracranial infections or intraventricular haemorrhage, the composition of CSF is significantly altered.6 CSF biochemistry and cytology reflect the nature and severity of the infection with elevated white cell count and reduced CSF glucose. Anaerobic metabolism of glucose results in lactate production, which interferes with CSF acidity. Lysis of cells releases proteins and other intracellular constituents, such as phosphorylated carbohydrates. As stated by Sauermann et al.,1 glucose-6-phosphate (G-6-P) is not detectable in human CSF under physiological conditions. Both aspects, namely CSF acidity and the release of phosphorylated carbohydrates, are of importance regarding the antimicrobial activity of fosfomycin: (i) fosfomycin activity is dependent on pH and its microbial killing is impaired at higher pH; and (ii) further, this substance utilizes the hexose phosphate transport system, which is inducible by G-6-P, as a way of entry into microorganisms.5 In our opinion, the ready availability of G-6-P can be regarded as an important biological determinant for the efficacy of fosfomycin in vivo. However, this assumption requires empirical proof.

In our in vivo pharmacokinetic study, all patients suffered from haemorrhagic contamination of ventricular CSF due to primary disease and presented with ventriculitis.2 In view of the in vitro results presented by Sauermann et al.,1 and our in vivo observations, we have reason to assume that the altered biochemical characteristics of the haemorrhagic and inflamed CSF seen in our patients indeed enhanced the antimicrobial activity exerted by fosfomycin under pathological conditions in human CSF. Based on these data, we are still convinced that co-administration of fosfomycin could represent an appropriate treatment option for nosocomial device-related intracranial infections with susceptible S. aureus isolates, especially in patients with allergy or intolerance to first-line therapy.1 Importantly, however, with the continuing emergence of multidrug-resistant staphylococci as frequent causes of nosocomial ventriculomeningitis in the neurocritical care patient population, treatment with novel antistaphylococcal agents with adequate CSF penetration, such as the oxazolidinone linezolid, should be considered.5

Transparency declarations
None to declare.

References

J Antimicrob Chemother 2010
doi:10.1093/jac/dkq215
Advance publication 11 June 2010