Interference of Asfotase Alfa in common immunoassays using ALP detection systems

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Introduction

➢ Hypophosphatasia (HPP) is the result of low activity of the tissue-nonspecific alkaline phosphatase (TNSALP), an enzyme that plays a role in bone mineralization and affects the development of bones and teeth[1,2].

➢ Asfotase alfa (AA, STRENSIQ®, Alexion Pharmaceuticals, Inc.) is the first FDA-Approved treatment for patients with perinatal-, infantile- and juvenile-onset hypophosphatasia[3].

➢ Reported falsely elevated cardiac Troponin I, β-hCG and testosterone concentrations measured in samples with elevated TNSALP with the Beckman Coulter DxI 800, which use alkaline phosphatase (ALP) for signal amplification [4,5]

➢ ALP continues to be used as the measuring/signalling system in routine assays for proteins, hormones and other small molecules, albeit less and less commonly. Because it contains the ALP active site, AA is able to catalyse the substrate as the antibody-conjugated ALP would within an assay. Therefore, AA present in a patient's sample may generate a false positive or a false negative assay result.

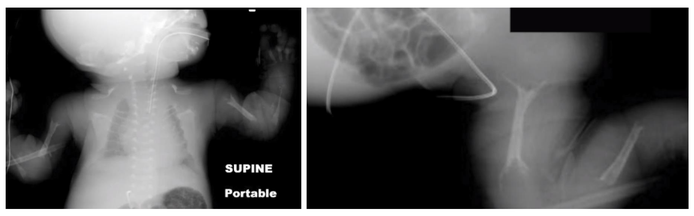


Figure 1: X-Rays of a child with HPP (extracted from a movie on HPP at http://alexion.com/patients

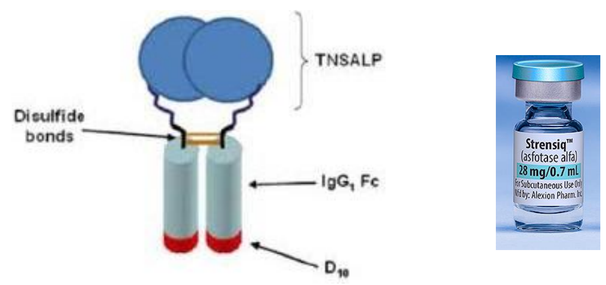


Figure 2: Structural characteristics of Asfotase Alfa (Strensiq™). AA is an enzyme containing the activity site of TNSALP.

Methods

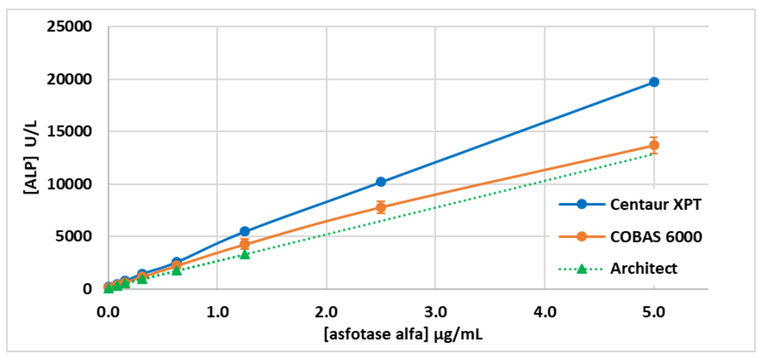
- Samples: Anonymized serum samples were provided by the Norwich and Norfolk University Hospital in accordance with general ethics guidelines.
- AA was added to the serum pool at concentrations from 0.08-5 µg/mL.
- Equipment: Siemens Immulite and ADVIA Centaur, Roche Diagnostics COBAS 6000 and Abbott Architect.
- Assays (Table 1) against TSH and fT4 were used using manufacturer's instructions. All experiments were repeated at least 3 times.
- Presence of the AA was confirmed by ALP measurement on the COBAS , Centaur and Architect.

Analyte	Assay type	Manufacturer	Detection system	
ALP	ADVIA Centaur XPT analyser	Siemens	ALP/pNPP	
	COBAS 6000 analyser	Roche Diagnostics	ALP/pNPP	
	Architect c16000 analyser	Abbott	ALP/pNPP	
TSH	Immulite 2000	Siemens	ALP/pNPP	
	ADVIA Centaur XPT analyser	Siemens	acridinium ester	
	COBAS 6000 analyser	Roche Diagnostics	ruthenium	
	Architect c16000 analyser	Abbott	Acridium ester	
fT4	Immulite 2000	Siemens	ALP/pNPP	
	ADVIA Centaur XPT analyser	Siemens	acridinium ester	
	COBAS 6000 analyser	Roche Diagnostics	ruthenium	
	Architect c16000 analyser	Abbott	Acridium ester	

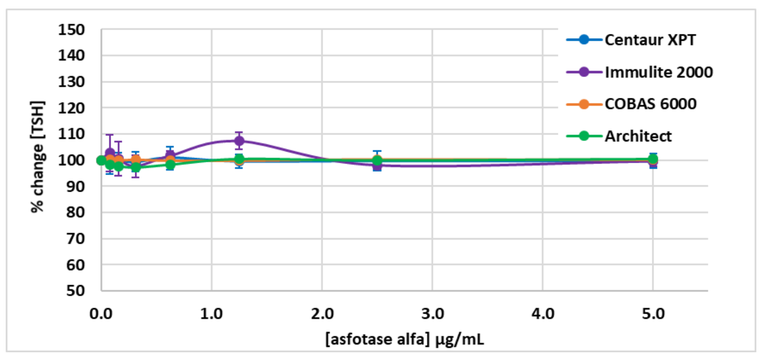
Table 1: Table showing the assay's characteristics.

Results

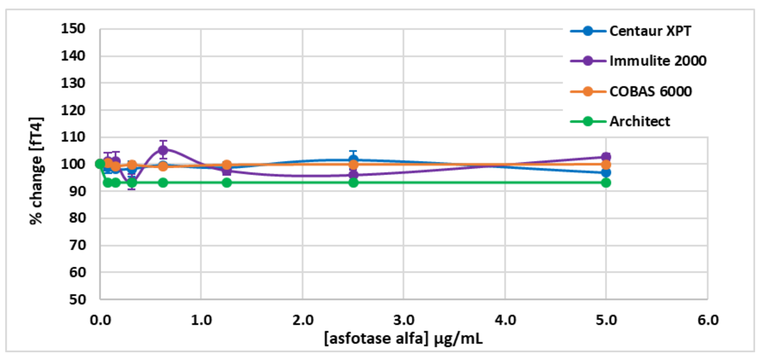
1- Asfotase alfa ALP activity can be measured in serum using ALP-assays (COBAS, Advia Centaur and Architect).



2- Asfotase alfa showed no interference effect on the measurements of TSH, whatever the equipment and the detection system



3- Asfotase alfa showed no interference effect on the measurements of fT4, whatever the equipment and the detection system



Conclusions

- In treatment of perinatal/infantile-or juvenile-onset HPP, the recommended dosage of asfotase alfa is 2mg/kg three times a week. A pharmacokinetics study on 38 HPP patients showed maximum blood concentrations Cmax of 1794 ± 690 ng/mL for patients under the age of 5 and 2108 ± 788 ng/mL for patients above the age of 5. We tested concentrations up to 15 times higher than these measured concentrations.
- We did not observe any interference of the AA in any of the assays tested, neither acridium ester nor ruthenium for signal amplification.
- Both assays for fT4 and TSH tested using ALP detection systems were sufficiently specific not to show interference with AA up to a serum concentration of 5µg/mL.
- Analyses of Troponin I, β-hCG and testosterone on the Siemens Immulite using ALP as detection system has not been assessed as yet.

TAKE HOME MESSAGE:

The presence of AA must be taken into consideration when analysing blood samples using certain assay technology to avoid any risk of misinterpretation of false positive/negative results. Consultants must communicate this information to the analytical lab who can opt for an adequate detection system (different than ALP).

References:

1.Whyte, Bone 2017; 102:15-25.2. Whyte, Nature Reviews Endocrinology 2016;12:233-46. 3. FDA - Food and Drug Administration 2015. 4. Herman *et al.*, Clin Biochem. 2016; 49:1118-21. 5. Sofronescu *et al.*, Clin Biochem. 2018; 58:118-121

Disclosures:

Alexion Pharmaceuticals, Inc. provided asfotase alfa and a medical review of the abstract for authors' consideration.