Moving to ICP-MS for sweat chloride determination – a review of patient outcomes

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Background and Objectives

Sweat chloride is the gold standard diagnostic marker for cystic fibrosis (CF), providing a direct functional measurement of the cystic fibrosis transmembrane regulator (CFTR). Recommended methods for quantification of chloride in sweat include: coulometry, direct and indirect ion selective electrodes, Colorimetry and Mercurimetric detection, however inductively-coupled plasma mass spectrometry (ICP-MS) is an emerging technology in this field. Having recently changed from coulometry to ICP-MS in our laboratory, this work looks at the differences between the two methodologies with respect to patient outcome data. We present a method comparison alongside a retrospective review of positive and borderline sweat chloride results.

Method

Patient sweat was collected using the Gibson and Cooke methodology and samples were eluted from filter paper into either 1% nitric acid (HNO_3) for ICP-MS analysis or 15 mM lithium nitrate buffer ($LiNO_3$) for analysis by Coulometry. Volunteers were sweated during the ICP-MS method validation in order to compare elution buffers as a direct patient comparison was not possible.

Sweat chloride data were analysed from April 2015 to March 2020 on subjects not referred by the NBS pathway. Patient outcome data were correlated for positive and intermediate sweat chloride results. Method comparison data was applied to all positive and borderline results to determine any potential changes in patient outcomes.

Results

Method comparison (ICP-MS vs coulometry)

ICP-MS demonstrated a constant negative bias (-2.244 mmol/L) compared with coulometry for sweat chloride measurements in EQA samples (Figure 1 A+B). Elution with HNO_3 showed a comparable constant negative bias (-2.714 mmol/L) compared with elution into $LiNO_3$ for sweat collected from volunteers and analysed by ICP-MS (Figure 1 C+D). Sweat chloride analysis using the coulometry method previously ran with a constant positive bias (3.4 mmol/L) compared to the ALTM on EQA (data not shown). Moving to ICP-MS has resulted in improved EQA performance with good agreement compared to both the ALTM and ICP-MS users. Sweat sodium showed excellent agreement between flame photometry and ICP-MS (data not shown). EQA performance is good and has remained unchanged since moving to ICP-MS analysis.

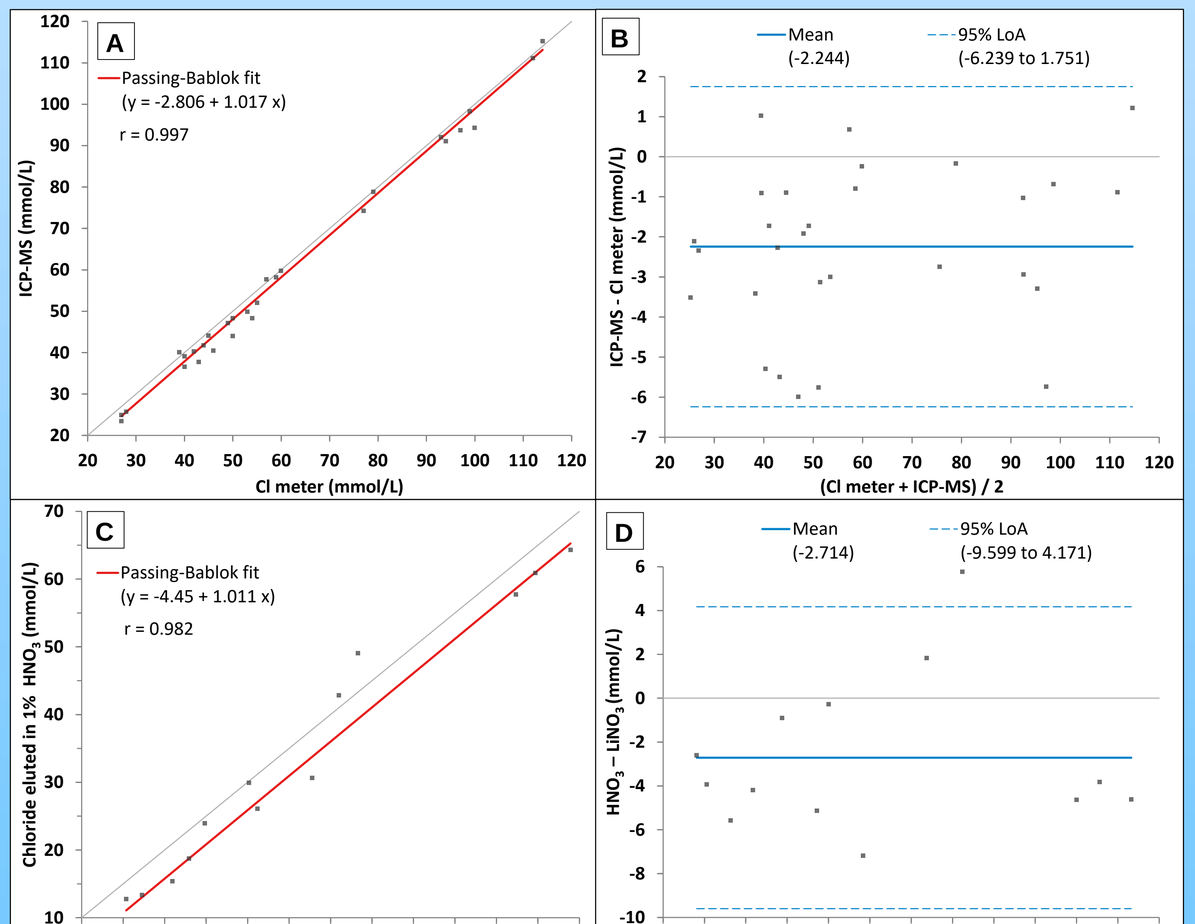

Figure 1 – Method comparison data. Passing Bablock and Bland Altman plots for: EQA samples (A and B) analysed by coulometry (eluted in $LiNO_3$) and ICP-MS (eluted in HNO_3) and ICP-MS analysis of sweat collections from volunteers (C and D) eluted in $LiNO_3$ and HNO_3.

ICP-MS analysis gives comparable precision compared with coulometry (Table 1), which meets the target specified by UK sweat testing guidelines of <5% CV at a chloride concentration of 50-60 mmol/L.[1]

	ICP-MS			Coulometry		
	Level 1	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**
n	160	165	160	30	30	29
Mean (mmol/L)	23.66	49.03	97.26	26.00	51.43	97.52
SD (mmol/L)	0.69	1.37	2.76	0.91	1.19	1.81
%CV	2.91	2.79	2.68	3.50	2.32	1.85

Table 1 – Precision data for the measurement of sweat chloride by ICP-MS and coulometry. Data gathered from routine operational use.

Review of patient outcomes

Sweat Chloride Results

Figure 2 shows all sweat chloride results obtained for 768 patients between April 2015 and March 2020 that were not referred via the NBS pathway. Final CF diagnosis was based on a combination of sweat chloride result, genetic testing and clinical review. Sweat chloride analysis changed over from coulometry to ICP-MS in November 2019, therefore most of these samples were analysed via coulometry. The median sweat chloride concentration was 76 mmol/L for patients diagnosed with CF (interquartile range 57 – 104 mmol/L). Despite the wide distribution of chloride results for CF affected patients, potentially due to varying disease severity, all recorded concentrations for this cohort were > 42 mmol/L. Sweat chloride concentrations were notably lower for patients unaffected by CF (median 16 mmol/L, interquartile range 13 – 21 mmol/L). A small number of CF unaffected patients (n = 11, 1.4 %) presented with borderline or elevated chloride concentrations. Sweat chloride concentrations for CF carriers ranged from 26 to 45 mmol/L.

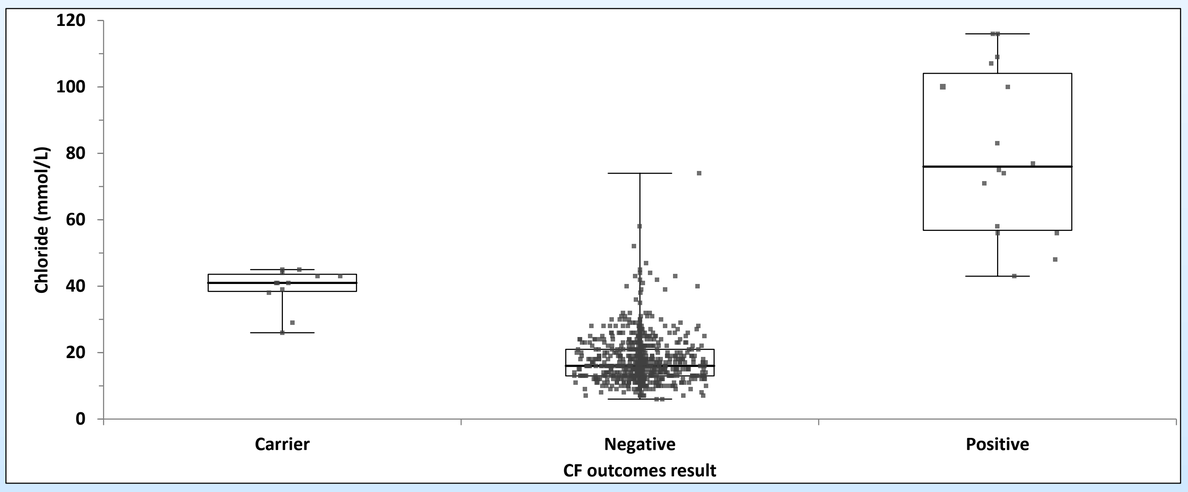

Figure 2 – Sweat chloride results for all patients presenting at RMCH April 2015 to March 2020 (median, interquartile range (box) and range (whiskers)).

Potential impact of the method change

Of the 768 patients that underwent sweat testing, 27 were new referrals to RMCH with borderline or positive sweat chloride results. Given the demonstrated bias between ICP-MS and coulometry, patient results within 3 mmol/L of clinical cut-offs were reviewed in relation to outcome data:

- 11 CF unaffected patients (1/11 had sweat chloride measured by ICP-MS)
  - 3/11 had reported sweat chloride concentrations less than 43 mmol/L measured by coulometry. Analysis by ICP-MS may have yielded results within the normal range.
- 5 patients diagnosed as CF carriers (all results measured by coulometry), of which 2 may have been missed using the ICP-MS method
  - 1 patient had an initial sweat chloride of 41 mmol/L. Repeat analysis yielded chloride concentrations below 30 mmol/L and genetic testing identified the patient as being a carrier of *CFTR* c.350G>A p.(Arg117His) in *cis* with T7, a variant associated with a range of *CFTR*-related phenotypes.
  - 1 patient had an initial sweat chloride of 38 mmol/L which repeated at 43 mmol/L. Genetic analysis showed this patient to be a carrier of the *CFTR* c.1521_1523delCTT p.(Phe508del) pathogenic variant.
- 4 patients with an inconclusive diagnosis – 2 results measured by coulometry and 2 by ICP-MS. Results likely unaffected by method change.
- 7 patients diagnosed as CF positive by coulometry – results likely unaffected by method change.

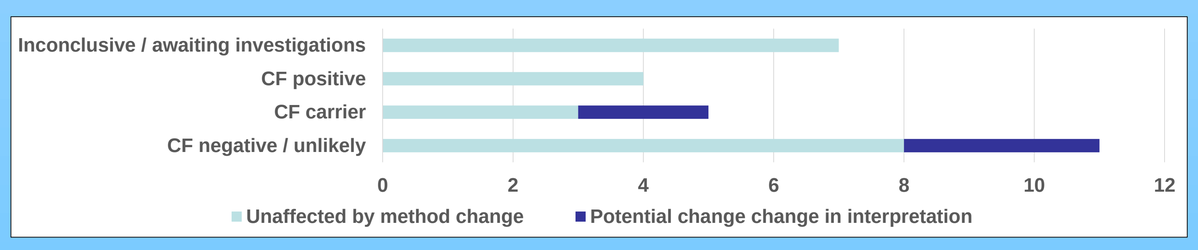

Figure 3 – Bar chart demonstrating the potential impact of changing methods on clinical interpretation for the 27 patients newly presenting to RMCH.

Conclusions

ICP-MS demonstrated improved accuracy (in relation to EQA performance) and comparable precision to coulometry for sweat chloride analysis.

This review of clinical outcomes demonstrates the overlap in sweat chloride concentrations in unaffected individuals and CF carriers. We highlight the importance of correlating clinical review with analytical results, especially around clinical cut-offs and for disorders such as CF where there is wide phenotypic and genetic variation. Whilst moving to ICP-MS may have prevented unnecessary follow up in 3 patients, appropriate follow up could have been missed in 2 cases had clinicians rigidly stuck to cut-off concentrations and not used clinical judgement.

References

1. Guideline for the performance of sweat test for the investigation of CF in the UK v2 (March 2014)
2. P021 Positive and borderline sweat test results for patients not referred via the newborn screening pathway: a review of patient outcomes. Robson A *et al*. Journal of Cystic Fibrosis, 2020-06-01, Volume 19, Pages S60-S61.