Review

The diagnostic power of salivary electrolytes for Sjögren's disease: a systematic literature review and meta-analysis

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ABSTRACT

Objective. *To perform a systematic review and meta-analysis to determine the power of salivary electrolytes for the diagnosis of Sjögren's disease (SjD).*

Methods. *A literature search was conducted (last search March 2023) using PubMed and Web of Science and completed with a manual search. Articles were screened for reports of human salivary ion concentrations, comparing SjD patients with healthy controls and/or sicca patients. Articles not using the SjD classification criteria or performing the classification as part of the experimental design were excluded. Forest plots were used to present the meta-analyses results for each ion, distinguishing between salivary type (unstimulated and stimulated whole saliva, submandibular/sublingual and parotid saliva).*

Results. *A total of 21 out of 722 articles were eligible for inclusion. For SjD patients a significant increase in salivary ion concentration was observed for sodium, chloride and calcium when comparing to healthy controls. Significant differences between SjD and sicca patients were noted for sodium, chloride, phosphate, calcium, phosphate, nitrite and nitrate. Stimulated whole saliva showed larger variability in results between studies in comparison to other types of saliva (unstimulated whole saliva, submandibular/sublingual saliva and parotid saliva).*

Conclusion. *Despite differences in saliva type, salivary ion levels could be utilised for the screening for SjD. Making use of chloride in combination with sodium would be most promising for distinguishing SjD patients from healthy controls and adding phosphate to potentially make a distinguishment with sicca patients. Unstimulated whole saliva should be the first choice when testing salivary ion concentrations.*

Introduction

Sjögren's disease (SjD) is a chronic auto-immune disease for which the exact pathological mechanism remains unclear. Characteristic for SjD patients is lymphocytic infiltration of the exocrine glands with inflammatory responses and a loss of function of the glands. The disease can be classified into primary SjD (pSjD) and secondary/associated SjD (sSjD) in which it is associated with another autoimmune disease. Currently, the diagnosis of pSjD is done making use of the 2016 ACR-EULAR classification criteria as a guideline (1). Unfortunately, clinical representation differs greatly between patients, which often leads to difficulties and diagnostic delays (2, 3). Furthermore, for sSjD no established classification exist (4).

Because common hallmarks for SjD are fatigue and oral and ocular dryness, both saliva and tears can be used as a non-invasive matrix for the detection of SjD related biomarkers (5, 6). Considering, that saliva has a wide array of components such as ions and proteins to asses as biomarkers, it is of interest to further explore its relation to SjD (5, 7, 8). Salivary proteomics has recently been systematically reviewed for its use in SjD diagnostic work-up, classification and progression, and revealed that there are striking differences in the levels of certain salivary proteins (9). However, to date, a systematic review of the literature on the differences in salivary electrolytes between SjD patients and healthy controls and sicca patients and whether there is potential for diagnosis/classification is still missing. This systematic review aims to deter-

mine the power of salivary electrolytes for the diagnosis/classification of SjD.

Methods

Protocol registration

The research protocol was registered in the international Prospective Register of Systematic Reviews (PROSPERO; CRD42023396071). An amendment to the protocol was made on the 5th of June 2023. The guidelines as described in the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 Statement were followed (Supplementary Table S1) (10).

Study selection

Article eligibility was defined based on the PICO tool (11). Population (P): Adult humans (age: ≥18); Intervention/Exposure (I): SjD as defined by the criteria available at the time of publication of the included articles; Comparison (C): Healthy controls or non-SjD patients; Outcome (O): Difference in salivary ion concentrations between the groups. Only original articles written in English describing ion concentration in any type of human saliva were considered eligible (last search March 2023). Exclusion criteria included:

- Articles not describing which classification and/or diagnostic criteria were followed to determine/define SjD (1, 12-14);
- Articles in which the classification and/or diagnostic procedure for SjD was executed as part of the experimental design;
- Articles without salivary ion concentrations;
- Review articles, opinion articles, letters to the editor, case reports and articles describing animal experiments.

Search strategy

The initial literature search was conducted by two examiners (JSS, ZA) independently, in PubMed and Web of Science. The search queries used to search for potential articles in each database are described in Supplementary Table S2. In addition, a manual search was conducted by screening the reference list and "Cited by" records of the resulting articles. For all potentially eligible articles, based on the title, abstract and methods, the full text was obtained. If not available, the authors were contacted to obtain the full text. All potentially eligible articles were screened by the original and an additional examiner (JSS, ZA, SAP). The manual search was also performed by two examiners (JSS, ZA) and confirmed by a third examiner (SAP). Any discrepancies were resolved through open discussion until an agreement was reached.

Data extraction

From the selected articles the following study characteristics were extracted:

- Study design: year of publication, demographics, saliva collection method, time of saliva collection, type of saliva collected, technique for ion concentration measurements;
- Population characteristics: diagnostic/classification criteria, type of saliva analysed, age, male/female ratio;
- Outcome measures: flow rate (FR), ion concentrations.

Data extraction was performed by two examiners (JSS, ZA) independently. In case of missing data, multiple attempts were made to contact the authors, with a response time of up to 17 weeks. Without a response and no data on the ion concentrations were available, then the article was excluded. In case of any data reported in a different format, such as for the concentration of the outcome measurements, then the results were converted to the concentration in mM and for the nitrite measurements in μM. Whenever the median and range/interquartile range were reported, then the mean and standard deviation (SD) were imputed as previously described (15, 16).

Risk of bias assessment

To assess for the potential risk of bias, the quality of the articles was evaluated. The Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies was used (17). The final quality rating was awarded based on the total number of YES per article. The total possible score was calculated based on the total number of questions and subtracting the criteria which were not applicable, resulting in a total possible score of 12. Quality ratings being: "Poor" (1–4), "Fair" (5–8) and "Good" (9–12). The quality assessment was conducted by 2 examiners (JSS, ZA) independently and any discrepancies were resolved through open discussion until a consensus was reached. In case of continued disagreement, additional opinions were requested from other researchers (FJB, MLL, SAP) to settle the discussion. If a question could not be rated, due to the inaccessibility of cited references, the author of the article was contacted. In case of no reply, the respective question was marked with CD (cannot be determined).

Quantitative analysis

To assess the differences in ion concentrations between SjD patients and healthy controls across the different types of saliva, a quantitative analysis was performed. Ions to be assessed, were those presented in the included articles, namely: sodium, chloride, phosphate, calcium, potassium, magnesium, nitrite and nitrate. Data was further separated based on the three possible patient groups (subgroups): pSjD patients, sSjD patients and a group combining both (SjD patients). Whenever sufficient data for an ion, within the same type of saliva was available for at least two subgroups (SjD, pSjD or sSjD), a subgroup meta-analysis was performed. Differences between the groups were analysed using Q_M statistics to determine the heterogeneity with the different types of SjD patients assigned as a moderator. Whenever data was available for only one SjD patient group, a singular meta-analysis was executed. All meta-analyses and statistics were performed using R (18) in Rstudio (19) with the "metafor" (20), "dplyr" (21) and "tidyverse" (22) packages. The mean difference (MD) with a 95% Confidence Interval (95% CI) was determined making use of a Random Effects Model. Measures of heterogeneity for the subgroups and the combined subgroups include Tau^2 (τ^2) as a measure of dispersion of the true effect size between studies, the Q-statistic (Q) to measure the differences between observed effects and the weighted average effect and I^2 to depict the proportion of observed variance which reflects the real difference in effect size. The height of I^2 was determined as follows: 0–30%,

low heterogeneity; 30–50%, moderate; 50–75%, substantial; and 75–100%, considerable. For the interpretation of the effect size, the z-statistic (z) was used, depicting the effect size for the overall meta-analyses, including *p*-value. The significance level was set at α=0.05. Forest plots were used to visualise the obtained data from the meta-analyses. For studies making use of the same healthy control group, per (subgroup) meta-analyses the study population size (N) was evenly divided over all the studies making use of the aforementioned group.

Data synthesis

To determine whether there is a change in absolute quantities of salivary ions between SjD patients and healthy controls the output was estimated (in mmol/min). This was done by making use of the mean FR and mean ion concentration data for each article if mentioned. Figures were generated making use of GraphPad Prism 8 (v. 8.0.2.263 for Windows).

Results

Search results and study selection

A total of 722 records fulfilled the search criteria and were screened (Fig. 1). After reviewing the records, 672 were excluded based on title and abstract. From the 50 potential eligible records, 17 duplicates were removed. The manual search resulted in 5 additional records. 17 records were excluded for various reasons extensively described in Supplementary Table S3. At conclusion of screening, 21 articles were found to be eligible for review.

Description of studies

All included studies were cross-sectional case-control studies, except for a single observational cohort study (Table I). Seven studies used the 2002 American-European Classification criteria (12) and one used the 2016 ACR-EULAR criteria (1) to classify SjD. The other articles used criteria or standards eligible at the time of publishing. Most studies tested multiple types of saliva: 6 articles described results on unstimulated whole saliva (UWS), 8 on stimulated whole saliva (SWS), 5 on submandibular/sublingual (SM/SL) saliva and 12 on parotid saliva. Ten studies collected saliva samples in the morning, of which 8 collected it between specific hours. Four studies collected the samples at specific time point in the afternoon, 3 studies at variable times of which 2 after one hour of fasting. Three studies did not report the collection time. The most common technique for measuring the electrolyte concentration was (flame) photometry (10 studies). Other measurement techniques included (atomic absorption) spectroscopy (7 studies), spectrophotometry (7 studies), (silver ion) titration (6 studies), colorimetry (4 studies), phosphate reduction assays (3 studies), the modified Griess method (1 study), latex agglutination assay kit (1 study) and high-performance liquid chromatography (HPLC) (1 study).

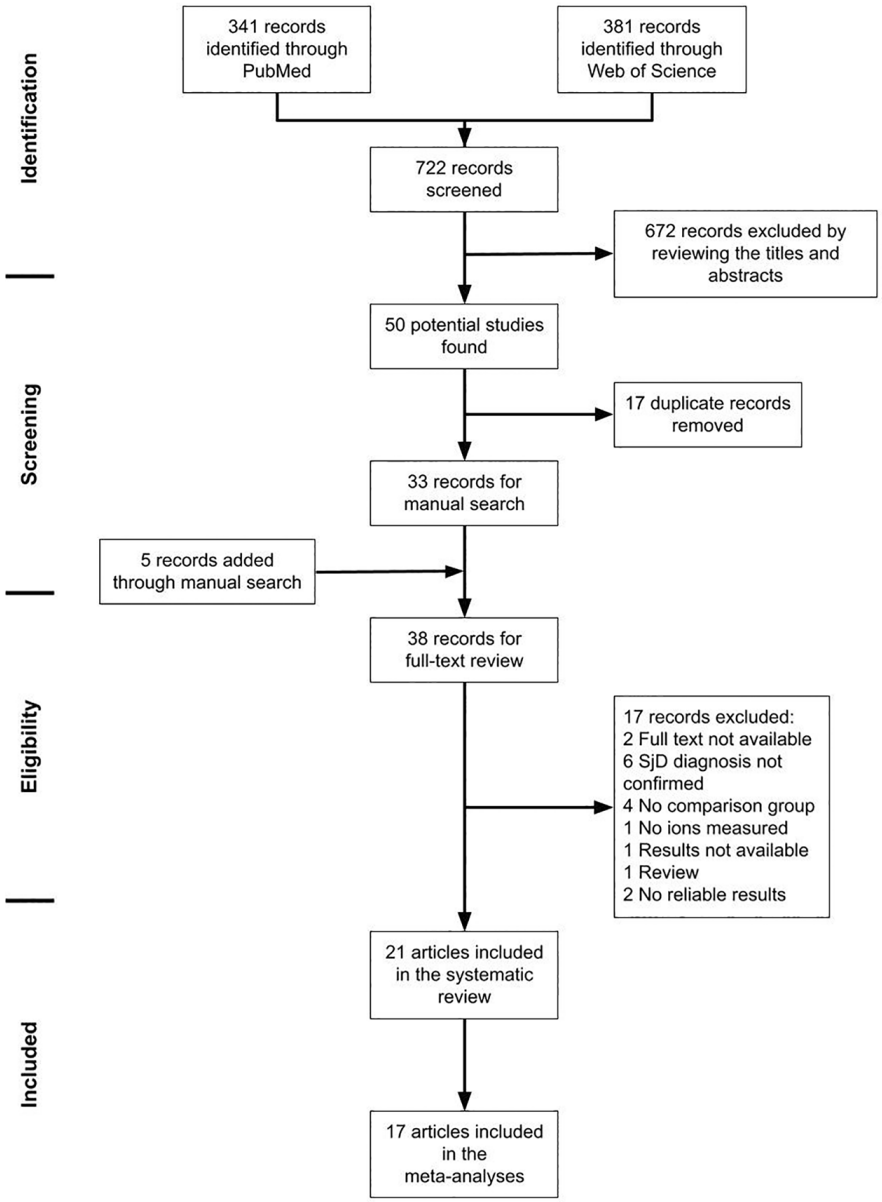

Fig. 1. PRISMA flow chart for the selection of the eligible articles.

Risk of bias assessment

The risk of bias assessment for the 20 selected articles is presented in Supplementary Table S4. Six articles were rated as "Poor", 14 as "Fair" and 1 as "Good", with an overall study quality score of "Fair". These scores give an indication of the potential bias that could be attributed to how the studies were reported, regardless of whether additional information could be obtained. Studies with a "Poor" quality assessment score were

Table I. Characteristics of the included studies.

Authors, year	Study type	Country	Patient groups (N)	SjD classification	Type of saliva collected	Saliva collection method	Time of saliva collection	Ions measured	Measuring technique
Almståhl & Wikström, 2003 (23)	Cross-sectional case control study	Sweden	Healthy control (12), pSjD (10), radiotherapy (9), xerostomia patients of unknown cause (10)	Copenhagen criteria (14)	SWS	Drooling/ passive	8:00-12:00, sober for 2h	Na^+, Ca^{2+}, K^+, PO_4^{3-}	Flame photometry, photometry at 340 nm
Ancuta *et al*., 2017 (24)	Cross-sectional case control study	Romania	Healthy control (10), pSjD (10)	2002 American-European classification consensus (12)	SWS	Spitting/ active	9:00-10:00, sober for 2h	Na^+, Cl^-, K^+	Automated Analyser
Asashima *et al*., 2013 (25)	Cross-sectional case control study	Japan	Healthy control (75), pSjD (71), sSjD (50), other connective tissue disease patients (20)	2002 American-European classification consensus (12)	UWS	NR	Morning, sober for 2h	Na^+, Cl^-, K^+	Latex agglutination assay kit
Atkinson *et al*., 1990 (26)	Cross-sectional case control study	USA	Healthy control (25), pSjD (64)	Schirmer tear test, salivary gland biopsy, at least one serological alteration	Stimulated parotid + SM/SL	Suction	8:30-11:30, sober for 1.5h	Na^+, Cl^-, K^+	Atomic absorption spectroscopy, colorimetry
Ben-Aryeh *et al*. 1981 (27)	Cross-sectional case control study	Israel	Healthy control (15), pSjD (22), sSjD (12), sicca patients (14)	Xerostomia, positive Schirmer test and positive rose bengal staining of the cornea and sSjD in the presence of rheumatoid arthritis (28)	UWS	CD	CD	Na^+, K^+	Flame photometry
Benchabane *et al*., 2016 (29)	Cross-sectional case control study	France	Healthy control (15), pSjD (44)	2002 American-European classification consensus (12)	SWS	NR	After an overnight fast	NO_2^- (as a measure for NO)	Modified Griess method
Benedek-Spät *et al*., 1975 (30)	Cross-sectional case control study	Hungary	Healthy control (32), SjD (13)	Lympho-epithelial lesions to the parotid gland (biopsy) or keratoconjunctivitis sicca, xerostomia and rheumatoid arthritis	Stimulated parotid	Suction	8:00-9:00, fasting state	Na^+, PO_4^{3-}, K^+	Flame photometry
Kalk *et al*., 2001 (31)	Cross-sectional case control study	the Netherlands	Healthy control (36), pSjD (33), sSjD (25), non-SjD rheumatology patients (42)	1993 Preliminary European classification criteria (13)	Stimulated parotid + SM/SL	NR	13:00-15:00, sober for 1.5h	Na^+, Cl^-, PO_4^{3-}, Ca^{2+}, K^+	Flame photometry, titration, spectrophotometry, phosphate reduction assay
Kalk *et al*., 2002 (32)	Cross-sectional case control study	the Netherlands	pSjD (32), sSjD (25), non-SjD patients (23)	2002 American-European Classification Consensus (12)	Stimulated parotid	NR	13:00-15:00, sober for 1.5h	Na^+, Cl^-, PO_4^{3-}	Flame photometry, silver ion titration, spectroscopy
Mandel & Baurmash, 1976 (33)	Cross-sectional case control study	USA	Healthy control (12), SjD (12)	Systemic involvement, labial gland biopsy & sialography	Stimulated parotid	Suction	NR	Na^+, Cl^-, PO_4^{3-}, Ca^{2+}, K^+	Atomic absorption spectrophotometry, colorimetry
Miller *et al*., 2012 (34)	Cross-sectional case control study	Israel	Healthy control (24), SjD (17)	2002 American-European classification consensus (12)	UWS	Spitting/ Active	10:00-12:00, NR	Na^+, Cl^-, PO_4^{3-}, Ca^{2+}, K^+, Mg^{2+}	Olympus Au680 clinical chemistry analyzer
Nahir *et al*., 1987 (35)	Cross-sectional case control study	Israel	Healthy control (20), SjD (9), rheumatoid artthritis (11)	Classical or definite rheumatoid arthritis, with xerostomia, positive Schirmer test and positive rose bengal staining of the cornea	UWS + SWS	Spitting/ Active	Same time of day, sober for 1h	Na^+, K^+	Flame photometry

Authors, year	Study type	Country	Patient groups (N)	SjD classification	Type of saliva collected	Saliva collection method	Time of saliva collection	Ions measured	Measuring technique
Pedersen *et al*., 1999 (36)	Cross-sectional case control study	Denmark	Age-matched healthy control (14), younger healthy control (13), pSjD (16)	1993 Preliminary European classification criteria (13)	UWS + SWS + stimulated parotid	NR	9:00-11:30, NR	Na^+, K^+	Atomic absorption spectrophotometry
Pedersen *et al*., 2005 (37)	Cross-sectional case control study	Denmark	Healthy control (20), pSjD (20)	2002 American-European classification consensus (12)	UWS + SWS	NR	9:00-11:45, sober for 2h	Na^+, Ca^{2+}, K^+	Atomic absorption spectrophotometry, colorimetry
Pijpe *et al*., 2007 (38)	Observational cohort study	the Netherlands	Healthy control (36), pSjD (32), sSjD (28)	2002 American-European classification consensus (12)	Stimulated parotid + SM/SL	Suction	13:00-15:00, sober for 1.5h	Na^+, Cl^-, K^+	Flame photometry, titration, spectrophotometry, phosphate reduction assay
Pringle *et al*., 2021 (39)	Cross-sectional case control study	the Netherlands	pSjD (47), non-SjD metabolic (16), medication (8) and other sicca patients (41)	2016 ACR-EULAR classification criteria (1)	Stimulated parotid + unstimulated/ stimulated SM/SL	NR	Sober for 1h	Na^+, Cl^-, PO_4^{3-}, K^+	Flame photometry, spectrophotometry, titration with silver ions
Stuchell *et al*., 1984 (40)	Cross-sectional case control study	USA	Healthy control (12), SjD (15)	Dry mouth, with grade 4 lymphocytic infiltration of the minor salivary glands	Parotid (did not distinguish between stimulated and non-stimulated)	Suction	NR	Na^+, Cl^-, PO_4^{3-}, Ca^{2+}, K^+	Atomic absorption spectrophotometry, colorimetry
van den Berg *et al*., 2007 (41)	Cross-sectional case control study	the Netherlands	Healthy control (36), SjD (62), sialosis (45), medication induced (9) and sodium retention syndrome patients (30)	2002 American-European classification consensus (12)	Stimulated parotid	Suction	13:00-15:00, sober for 1.5h	Na^+, Cl^-, K^+	Flame photometry, titration, spectrophotometry, phosphate reduction assay
van der Reijden *et al*., 1996 (42)	Cross-sectional case control study	the Netherlands	Healthy control (17), pSjD (33), sSjD (10)	1993 Preliminary European classification criteria (13)	SWS	NR	10:00-16:00	PO_4^{3-}, Ca^{2+}	Atomic absorption spectroscopy, method by Chen *et al*. (43)
Vissink *et al*., 1993 (44)	Cross-sectional case control study	the Netherlands	Healthy control (36), SjD (23)	1993 Preliminary European classification criteria (13)	Stimulated parotid + SM/SL	Suction	NR	Na^+, Cl^-, Ca^{2+}, K^+	Flame photometry, silver titration, spectrophotometry at 577 nm and 600 nm
Xia *et al*., 2003 (45)	Cross-sectional case control study	China	Healthy control (29), SjD (31), sialosis (30)	1993 Preliminary European classification criteria (13), salivary flow rate (46), parotid swelling (47)	SWS + stimulated parotid	Spitting/ Active & Suction	8:00-10:00, fast overnight and no brushing 8h	NO_2^-, NO_3^-	HPLC

N: number of patients included; SjD: Sjögren's disease; pSjD: Primary Sjögren's disease; sSjD: Secondary Sjögren's disease; UWS: unstimulated whole saliva; SWS: stimulated whole saliva; SM/SL: submandibular/sublingual; NR: not reported; CD: cannot be determined.

not excluded, but should be reviewed and analysed with some level of caution.

Patient characteristics

Table II describes the characteristics of the patients included. A total of 489 healthy controls, 182 SjD patients, 434 pSjD patients, 150 sSjD patients and 308 non-SjD dry mouth (sicca) patients were included. Average SjD disease duration reportedly ranged from 3–7 years overall. There was a wide variety in the causes for the dry mouth complaints of the sicca patients included in the studies: radiotherapy, receiving neuroleptic medication, connective tissue disease, rheumatoid arthritis, sialosis, medication-induced, metabolic disorder, sodium retention syndrome and patients with xerostomia with an unknown cause (and no salivary gland pathologies).

Table II. Patient characteristics per included article subdivided by the type of saliva.

Authors, year	Controls			pSjD			sSjD			Sicca patients		
	Age in years (mean±SD)	F/M	Flow rate ml/min (mean±SD)	Age in years (mean±SD)	F/M	Flow rate ml/min (mean±SD)	Age in years (mean±SD)	F/M	Flow rate ml/min (mean±SD)	Age in years (mean±SD)	F/M	Flow rate ml/min (mean±SD)
						UWS						
Asashima *et al*., 2013 (25)	50.7±15.6	43/32	0.38±0.42	60±16.8	69/2	0.06±0.14	55.8±17.4	49/1	0.09±0.30	60±16[d]	43/11[d]	0.16±0.29[d]
Ben-Aryeh *et al*., 1981 (27)	NR	NR	0.36±0.17	NR	NR	0.11±0.06	NR	NR	0.11±0.07	NR[c]	NR[c]	0.10±0.05[c]
Miller *et al*., 2012 (34)	49.3±9.8	24/0	0.49±0.19	55.5±15.7[k]	17/0[k]	0.12±0.08[k]	-	-	-	-	-	-
Nahir *et al*., 1987 (35)	NR	NR	0.25±0.04	NR[k]	NR[k]	0.06±0.06[k]	-	-	-	NR[e]	NR[e]	0.27±0.1[e]
Pedersen *et al*., 1999 (36)	50.8±9.8 24.1±3.7[f]	13/1 12/1[f]	0.44±0.25 0.30±0.17[f]	61.4±12.3 -	14/2	0.04±0.03	-	-	-	-	-	-
Pedersen *et al*., 2005 (37)	56.1±12.6	20/0	0.39±0.23	59.8±14.5	20/0	0.04±0.06	-	-	-	-	-	-
Van der Reijden *et al*., 1996 (42)	33.2±NR	NR	0.62±0.45	62.4±NR	NR	0.07±0.11	56±NR	NR	0.08±0.14	-	-	-
						SWS						
Almståhl & Wikström, 2003 (23)	54±8	10/2	2.39±0.81	57±10	10/0	0.52±0.20	-	-	-	55±6[a] 55±8[b] 54±8[c]	2/8[a] 4/6[b] 9/1[c]	0.53±0.36[a] 1.13±0.60[b] 1.10±0.40[c]
Ancuta *et al*., 2017 (24)	NR	NR	0.74±0.21	NR	NR	0.51±0.39	-	-	-	-	-	-
Benchabane *et al*., 2016 (29)	41.4±15.9	12/3	0.72±0.22	43.9±13.1	40/4	≤0.6±NR	-	-	-	-	-	-
Nahir *et al*., 1987 (35)	NR	NR	1.28±2.10	NR[k]	NR[k]	0.52±0.84[k]	-	-	-	NR[e]	NR[e]	1.03±0.70[e]
Pedersen *et al*., 1999 (36)	50.8±9.8 24.1±3.7[f]	13/1 12/1[f]	1.59±0.63 1.77±1.07[f]	61.4±12.3	14/2	0.64±0.45	-	-	-	-	-	-
Pedersen *et al*., 2005 (37)	56.1±12.6	20/0	1.40±NR	59.8±14.5	20/0	0.33±0.45	-	-	-	-	-	-
Van der Reijden *et al*., 1996 (42)	33.2±NR	NR	1.61±0.65	62.4±NR	NR	0.18±0.26	56±NR	NR	0.19±0.39	-	-	-
Xia *et al*., 2003 (45)	45±NR	14/15	1.86±0.84	48±NR[k]	29/2[k]	0.75±0.69[k]	-	-	-	46±NR[i]	11/19[i]	1.77±0.79[i]
						SM/SL						
Atkinson *et al*., 1990 (26)	46.7±NR	25/0	NR	55.7±NR	59/5	NR	-	-	-	-	-	-
Kalk *et al*., 2001 (31)	39±12	20/16	0.46±0.24	51±16	30/3	0.24 ±0.28	54±12	21/4	0.26±0.35	55±17[c]	40/2[c]	0.42±0.28[c]
Pijpe *et al*., 2007 (38)	39±12	20/16	NR	51±17	30/2	NR	55±13	23/5	NR	-	-	-
Pringle *et al*., 2021 (39)	-	-	-	53±14	45/2	0.16±0.19	-	-	-	47±11[g] 56±15[h] 48±13[c]	11/4[g] 7/1[h] 28/13[c]	0.2±0.14[g] 0.49±0.13[h] 0.31±0.2[c]
Vissink *et al*., 1993 (44)	NR	NR	0.46±0.24	45±NR[k]	21/2[k]	0.04±0.05[k]	-	-	-	-	-	-

Authors, year	Controls			pSjD			sSjD			Sicca patients		
	Age in years (mean±SD)	F/M	Flow rate ml/min (mean±SD)	Age in years (mean±SD)	F/M	Flow rate ml/min (mean±SD)	Age in years (mean±SD)	F/M	Flow rate ml/min (mean±SD)	Age in years (mean±SD)	F/M	Flow rate ml/min (mean±SD)
						Parotid						
Atkinson *et al*., 1990 (26)	46.7±NR	25/0	NR	55.7±NR	59/5	NR	-	-	-	-	-	-
Benedek-Spät *et al*., 1975 (30)	NR	NR	0.57±0.32	50±14.6[k]	10/3[k]	0.18±0.09 [k]	-	-	-	-	-	-
Kalk *et al*., 2001 (31)	39±12	20/16	0.52±0.42	51±16	30/3	0.12±0.13	54±12	21/4	0.24±0.25	55±17[c]	40/2[c]	0.19±0.15[c]
Kalk *et al*., 2002 (32)	-	-	-	53±14	30/2	0.13±0.15	58±13	19/6	0.15±0.19	48±12[c]	21/2[c]	0.19±0.12[c]
Mandel & Baurmash, 1976 (33)	NR	12/0	0.58±0.24	NR [k]	12/0[k]	0.17±0.10 [k]	-	-	-	-	-	-
Pedersen *et al*., 1999 (36)	50.8±9.8 24.1±3.7[f]	13/1 12/1[f]	0.44±0.39 0.47±0.36[f]	61.4±12.3	14/2	0.24±0.17	-	-	-	-	-	-
Pedersen *et al*., 2005 (37)	56.1±12.6	20/0	0.79±0.41	59.8±14.5	20/0	0.17±0.21	-	-	-	-	-	-
Pijpe *et al*., 2007 (38)	39±12	20/16	NR	51±17	30/2	NR	55±13	23/5	NR	-	-	-
Pringle *et al*., 2021 (39)	-	-	-	53±14	45/2	0.09±0.11	-	-	-	47±11[g] 56±15[h] 48±13[c]	11/4[g] 7/1[h] 28/13[c]	0.05±0.03[g] 0.19±0.08[h] 0.09±0.09[c]
Stuchell *et al*., 1984 (40)	NR	NR	0.58±0.07	59±NR[k]	NR[k]	0.31±0.27[k]	-	-	-	-	-	-
Van den Berg *et al*., 2007 (41)	39±12	20/16	0.52±0.42	NR[k]	NR[k]	0.15±0.18[k]	-	-	-	NR[i] NR[j] NR[h] NR[c]	NR[i] NR[j] NR[h] NR[c]	0.11±0.11[i] 0.24±0.15[j] 0.17±0.15[h] 0.19±0.19[c]
Vissink *et al*., 1993 (44)	NR	NR	0.52±0.42	45±NR[k]	21/2[k]	0.22±0.19[k]	-	-	-	-	-	-
Xia *et al*., 2003 (45)	45±NR	14/15	0.35±0.21	48±NR[k]	29/2[k]	0.08±0.03[k]	-	-	-	46±NR[i]	11/19[i]	0.26±0.14[i]

SD: standard deviation; F/M: female/male ratio; NR: not reported; UWS: unstimulated whole saliva; SWS: stimulated whole saliva; SM/SL: submandibular/sublingual.
[a]Radiotherapy in the head and neck region. [b]Patients receiving neuroleptic medication. [c]Xerostomia of unknown cause. [d]Patients with a connective tissue disease. [e]Patients with definite or classical rheumatoid arthritis. [f]Young healthy controls. [g]Patients with a metabolic disease/disorder. [h]Medication induced xerostomia. [i]Patients diagnosed as having sialosis on the basis of clinical and sialographical findings. [j]Sodium retention syndrome. [k]Unspecified SjD patients.

Data extraction and meta-analysis

Comparisons of the data were subdivided based on the type of ions measured, which saliva was used for the measurement (UWS, SWS, Parotid, SM/SL) and on whether the article included pSjD, sSjD or simply SjD patients. If both unstimulated and stimulated measurements were made for SM/SL and parotid saliva, then only the stimulated results were used for the meta-analysis. No meta-analysis was conducted for magnesium, nitrite and nitrate as an outcome measurement, due to a lack of data. The results for groups not included in a meta-analysis and the results comparing SjD patients and sicca patients, are reported separately in the Supplementary tables.

- *Sodium*. A total of 16 studies were included in the meta-analyses for sodium (Fig. 2). For UWS all subgroups display a significant increase, compared to healthy controls (combined MD=13.04mM; 95% CI=8.25–17.83; $p<0.001$). Despite substantial heterogeneity between the studies ($I^2=72.8\%$), there were no subgroup differences ($p=0.73$). SM/SL data, showed within subgroups (pSjD and sSjD) and combined an increase in sodium concentration (MD=6.18mM; 95% CI=3.54–8.83, $p<0.001$), compared to healthy controls. There are no subgroup differences ($p=0.09$) and heterogeneity between studies was low ($I^2=0.0\%$). For parotid an increase in sodium concentration was observed for pSjD and sSjD patients and all subgroups combined (MD=14.71mM; 95% CI=5.35–24.07; $p<0.01$), compared to healthy controls. Despite considerable heterogeneity between the studies ($I^2=91.4\%$), there are no subgroup differences ($p=0.68$). The 2 studies not included in the meta-analyses both reported a significant increase in sodium for SjD patients in

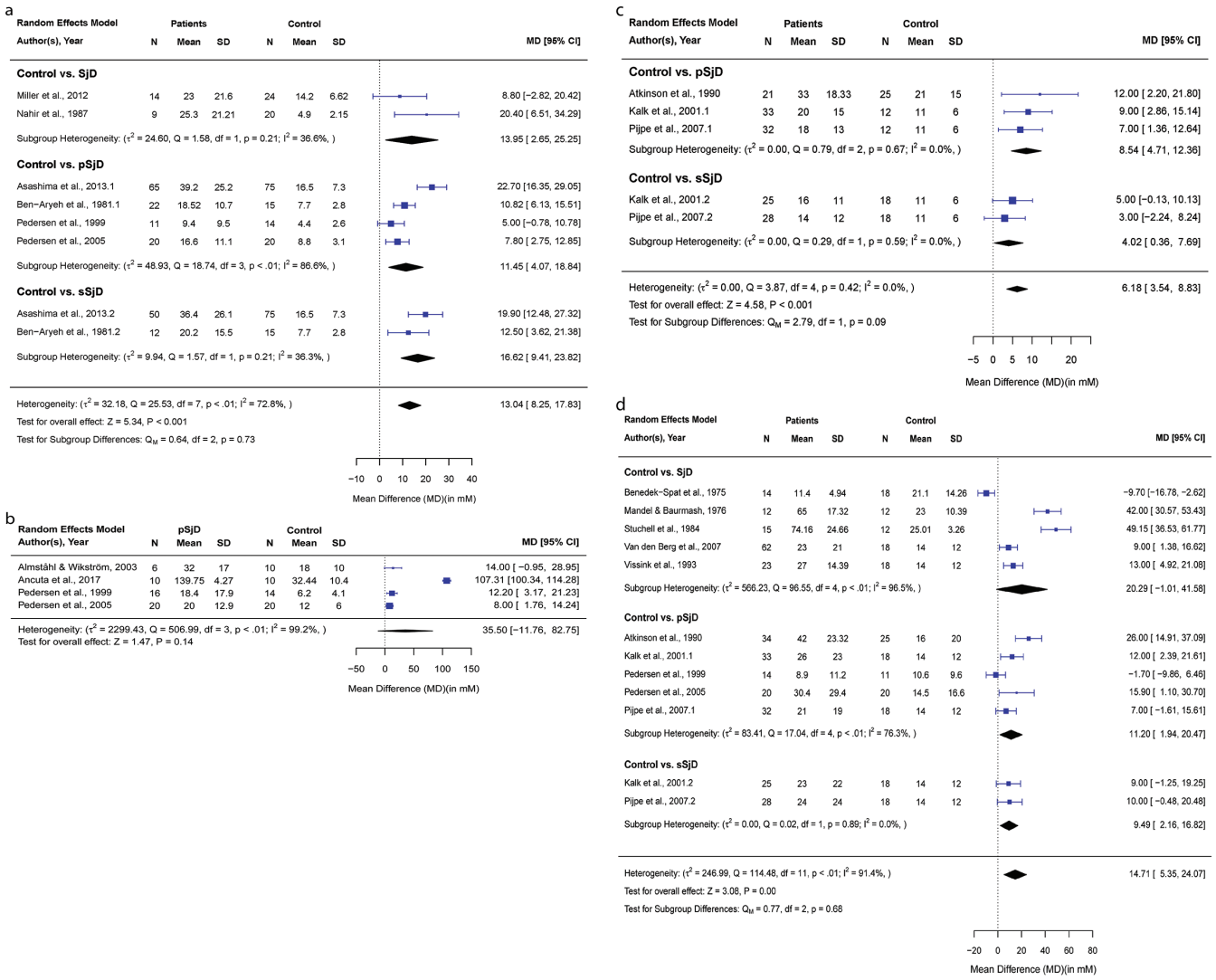

Fig. 2. Forest plot depicting the results of the meta-analyses for sodium concentration depicting the results in mean difference (in mM) for **a**) unstimulated whole saliva between SjD patients and healthy controls, primary SjD (pSjD) patients and healthy controls and between subgroups; **b**) stimulated whole saliva between SjD patients and healthy controls; **c**) submandibular/sublingual saliva between pSjD patients and healthy controls, secondary/associated SjD (sSjD) patients and healthy controls and between subgroups; and **d**) parotid saliva between SjD patients and healthy controls, pSjD patients and healthy controls, sSjD patients and healthy controls and between subgroups.
N: number of participants included; SD: standard deviation; 95% CI: 95% confidence interval.

SWS and SM/SL saliva respectively (Suppl. Table S5). The difference in sodium concentration between SjD patients and sicca patients was investigated by 8 studies, of which all but 1 reported a significant increase for SjD patients in UWS, SM/SL and parotid saliva (Suppl. Table S6).

- *Chloride*. A total of 10 studies were included in the various meta-analyses for chloride (Fig. 3). Despite a moderate heterogeneity (I^2=67.4%) between studies for UWS, an increase of chloride was found for pSjD patients compared to healthy controls (MD=19.99mM; 95% CI=11.47–28.50; p<0.001). For the SM/SL, there was an increase in chloride concentration for both subgroups (pSjD and sSjD) (combined MD=11.18mM; 95% CI=7.82–14.54; p<0.001). With no subgroup differences (p=0.28) and a low heterogeneity (I^2=0.0%). For parotid saliva there is also an overall increase in chloride for all SjD subgroups compared to healthy controls (MD=23.52mM; 95% CI=15.88–31.17; p<0.001). Despite a considerable heterogeneity between the studies (I^2=85.6%), there were also no subgroup differences (p=0.34). All 3 studies not included in the meta-analyses reported a significant increase of chloride concentration for SjD patients in UWS and SM/SL saliva (Suppl. Table S7). The difference in chloride concentration between SjD and sicca patients was described by 5 studies, of which 4 showed a significant increase in concentration for SjD patients in UWS, SM/SL and parotid saliva (Suppl. Table S8).

- *Phosphate*. Six studies were included in the various meta-analyses for phosphate comparing SjD patients to healthy controls (Suppl. Fig. S1). No statistically significant MDs were observed, probably attributed to the heterogeneity of the studies, ranging from moderate to considerable (I^2=70.6-97.2%). One out of 3 studies not in-

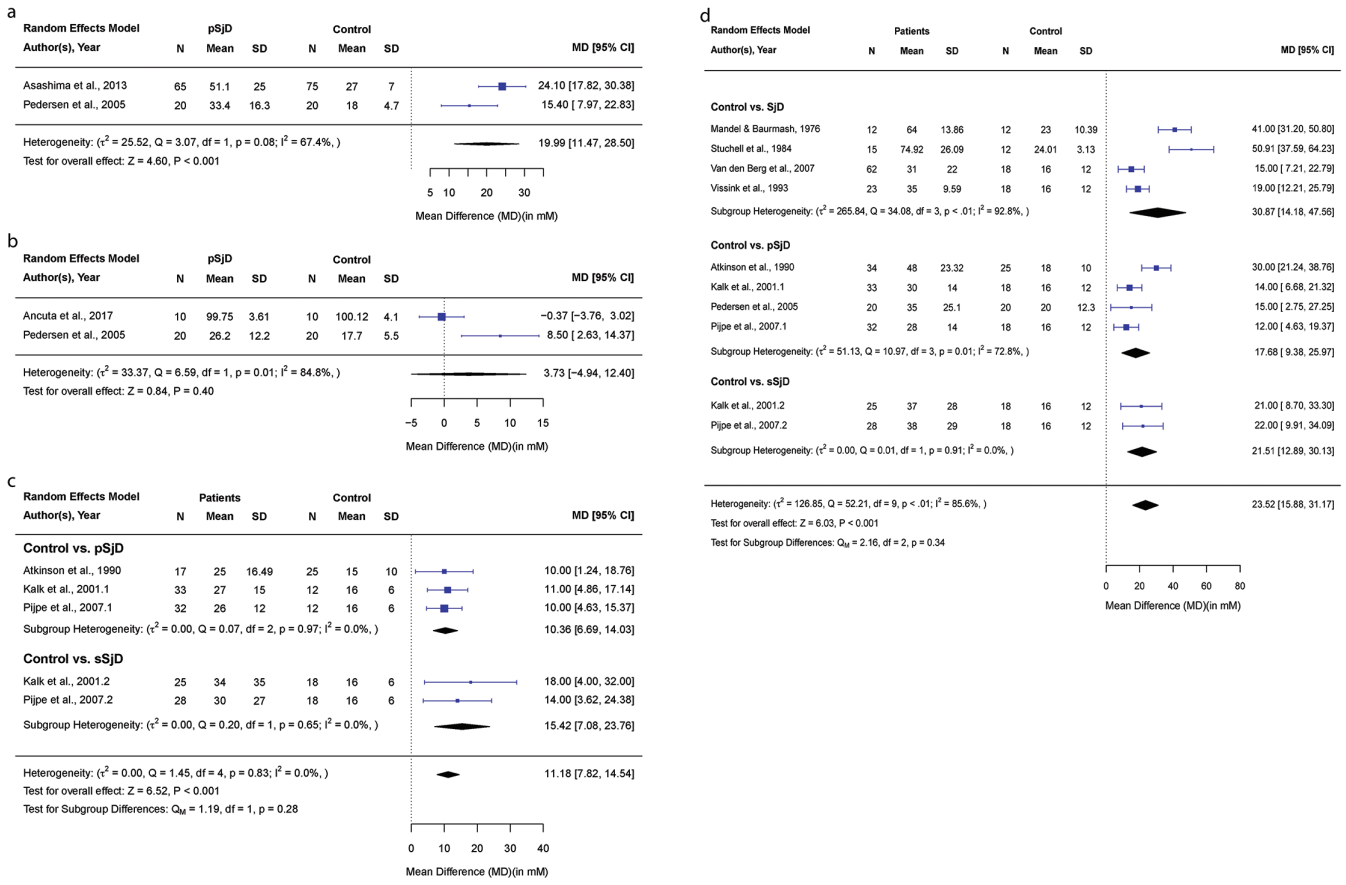

Fig. 3. Forest plot depicting the results of the meta-analyses for chloride concentration depicting the results in mean difference (in mM) for **a**) unstimulated whole saliva between primary SjD (pSjD) patients and healthy controls, **b**) stimulated whole saliva between pSjD patients and healthy controls, **c**) submandibular/sublingual saliva between pSjD patients and healthy controls, secondary/associated SjD (sSjD) patients and healthy controls and between subgroups and **d**) parotid saliva between SjD patients and healthy controls, pSjD patients and healthy controls, sSjD patients and healthy controls and between subgroups. N: number of participants included; SD: standard deviation; 95% CI: 95% confidence interval.

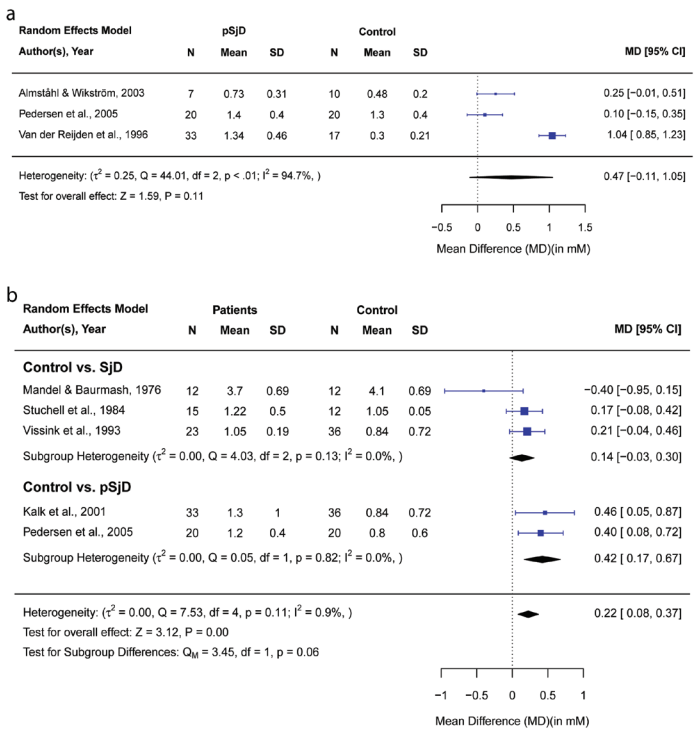

Fig. 4. Forest plots depicting the results of the meta-analyses for calcium concentration depicting the results in mean difference (in mM) for **a**) stimulated whole saliva between primary SjD (pSjD) patients and healthy controls; and **b**) parotid saliva between SjD patients and healthy controls, pSjD patients and healthy controls and between subgroups.
N: number of participants included; SD: standard deviation; 95% CI: 95% confidence interval.

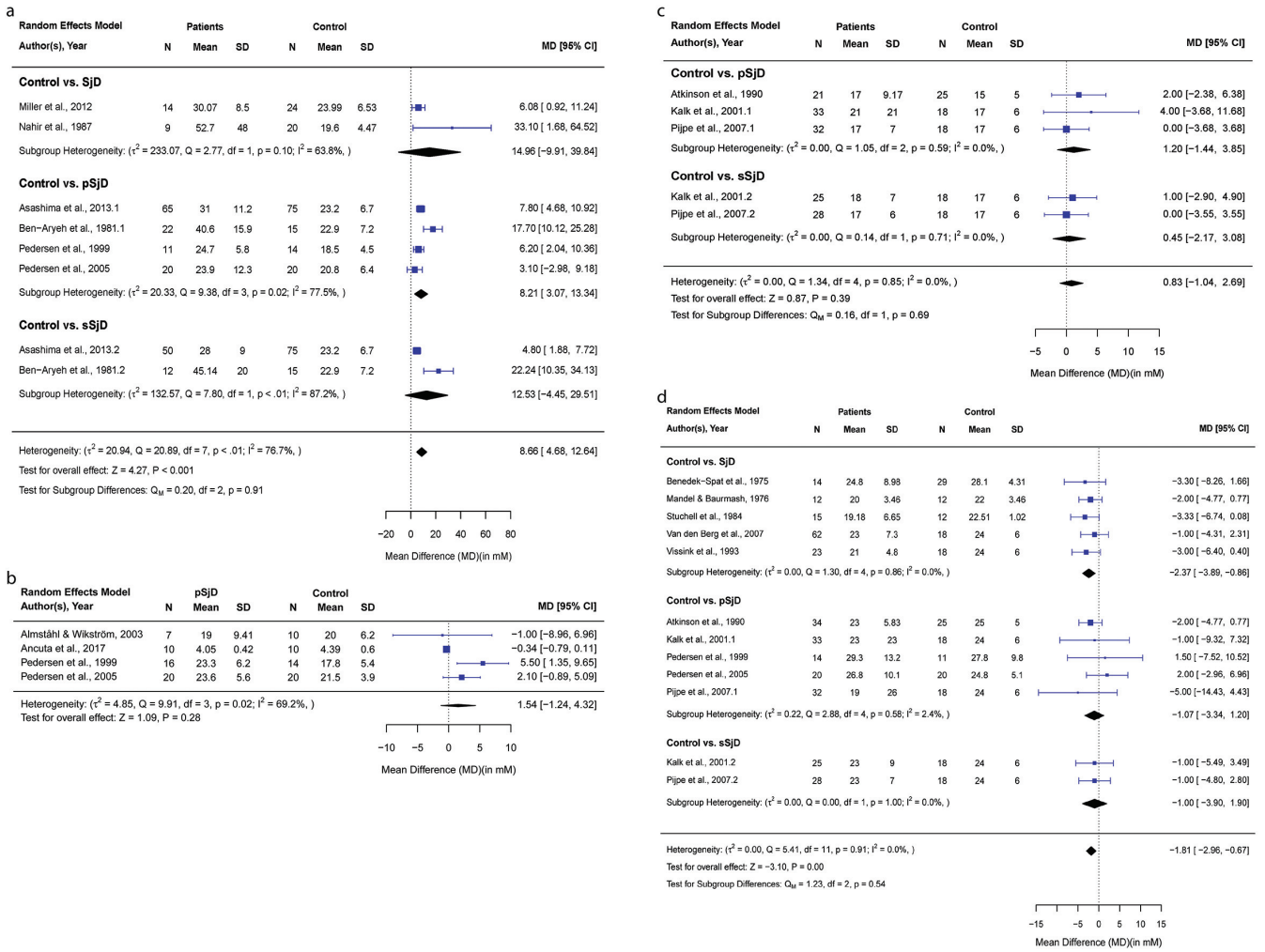

Fig. 5. Forest plot depicting the results of the meta-analyses for potassium concentration depicting the results in mean difference (in mM) for **a**) unstimulated whole saliva between SjD patients and healthy controls, primary SjD (pSjD) patients and healthy controls and between subgroups; **b**) stimulated whole saliva between SjD patients and healthy controls; **c**) submandibular/sublingual saliva between pSjD patients and healthy controls, secondary/associated SjD (sSjD) patients and healthy controls and between subgroups; and **d**) parotid saliva between SjD patients and healthy controls, pSjD patients and healthy controls, sSjD patients and healthy controls and between subgroups.
N: number of participants included; SD: standard deviation; 95% CI: 95% confidence interval.

cluded in the meta-analyses showed a significant increase for phosphate in SjD patients in SWS (Suppl. Table S9). From the 4 articles assessing the differences in phosphate concentration between SjD patients and sicca patients, all reported significant differences for SjD patients in SWS, SM/SL and parotid saliva (Suppl. Table S10).

- *Calcium*. A total of 7 studies were included in the various meta-analyses for calcium (Fig. 4). Higher calcium concentrations were found for parotid saliva for the pSjD patients, and when considering both subgroups (SjD and pSjD) together (MD=0.22mM; 95% CI=0.08–0.37; p<0.01), compared to healthy controls. There were no significant differences between the subgroups (p=0.06) and a low overall heterogeneity between studies (I^2=0.9%). Two out of five studies not included in the meta-analyses reported a significant increase in calcium concentration for SjD patients in UWS and SWS compared to healthy controls (Suppl. Table S11). No significant differences in calcium concentration between SjD patients and sicca patients were found in SWS, SM/SL and parotid saliva by the 2 studies investigating this (Suppl. Table S12).

- *Potassium*. A total of 16 studies were included in the meta-analyses for potassium (Fig. 5). In UWS, an increase in potassium, was observed for pSjD patients and all three subgroups combined (MD=8.66mM; 95% CI=4.68–12.64; p<0.001), compared to healthy controls. Despite considerable heterogeneity between the studies (I^2=76.7%) there were no subgroup differences (p=0.91). In contrast, in parotid saliva SjD patients had a decrease in potassium concentration and for all 3 subgroups overall (MD=-1.81mM; 95% CI=-2.96 – -0.67; p<0.01), compared to healthy controls. No differences between the subgroups (p=0.54) were found and a low heterogeneity between studies (I^2=0.0%). One study was not included in the meta-analyses, but showed a significant increase in potassium concentration for its SjD patient group in SWS (Suppl. Table S13). Differences in potassium concentration between SjD patients and sicca

Table III. Nitrite concentrations (mean ± standard deviation (SD)) in stimulated whole saliva (SWS) for the healthy controls and SjD patient groups and significant differences between them.

Authors, year	Control (µM)	SjD (µM)	Primary SjD (µM)
		SWS	
Bechabane *et al*., 2016 (29)	118.3±36.7	-	284.4±67.2*
Xia *et al*., 2003 (45)	226.06±126.07	147.81±71.73*	-

*Significant difference with the healthy control group as reported by the article.

Table IV. Nitrate concentrations (mean ± standard deviation (SD)) in stimulated whole saliva (SWS) and parotid saliva of SjD patients and healthy controls and the significant differences as reported by Xia *et al*., 2003 (45).

Saliva type	Control (mM)	SjD (mM)
SWS	1.56±0.76	0.39±0.24*
Parotid	2.77±1.32	0.85±0.55*

*Significant difference with the healthy control group as reported by the article.

patients were investigated by 7 studies, of which 5 reported significance in UWS, SWS and parotid saliva (Suppl. Table S14).

- *Magnesium*. Only one article investigated the concentration of magnesium between healthy controls and SjD patients in UWS (34). For the healthy controls they reported a mean of 0.3±0.21mM and for SjD patients 0.51±0.44mM, which was not significantly different.

- *Nitrite and nitrate*. No meta-analysis was performed for nitrite or nitrate as outcome measurements (Tables III and IV). Bechabane *et al*. reported a significant increase in nitrite concentration for pSjD patients in SWS, compared to healthy controls (29). Whereas, Xia *et al*. reported a significant decrease in nitrite and nitrate concentration for SjD patients compared to healthy controls in both SWS and parotid saliva (45). The latter also reported the differences between SjD patients and sialosis patients, showing a significant decrease in both nitrite and nitrate for SjD patients, in both SWS and parotid saliva (Suppl. Table S15).

Output estimates. An estimate of the output (in mmol/min) per ion was determined for each article per type of saliva. Figure 6 shows the relation between the sodium, chloride, phosphate, calcium and potassium output in parotid saliva against the average stimulated parotid FR, per patient group. SjD patients do not only have a lower FR, but also a relatively lower output of all the aforementioned ions. The data for the ions in other salivary types show a comparable trend (Suppl. Fig. S2-S4).

Discussion

The results of our meta-analyses show a wide interindividual variety in the levels of salivary ions in SjD, healthy controls and sicca patients, amongst different types of saliva. Notably, the meta-analyses showed a significant increase in sodium and chloride concentration for SjD patients compared to healthy controls and most sicca patients. Calcium was significantly increased for SjD patients compared to healthy controls, but not with sicca patients. For phosphate some individual articles showed a difference with various sicca patient groups.

Sodium and chloride were increased in UWS, SM/SL saliva and parotid saliva, but not in SWS of SjD patients. In healthy controls, there is normally an increase in sodium concentration upon stimulation. Nahir *et al*. showed that the opposite holds true for SjD patients, despite still having a higher sodium concentration in their SWS compared to healthy controls (35). Considering the function of sodium is to facilitate the production of saliva, this suggests a decrease in responsiveness to start this process. SWS ionconcentrations would therefore show greater variation. A similar trend could be expected for chloride, considering it is also involved in the facilitation of saliva secretion (48). Therefore, it is considered that perhaps more sodium and chloride is produced by the glands in SjD patients as a compensation for the loss of function (34). Alternatively, the resorption process, occurring in the glandular ducts, might be impaired (26, 39, 49). Limited, yet supporting evidence showed a positive correlation between lymphocytic infiltration and sodium channel disruption, through immunohistochemical staining of parotid gland biopsies (39). Furthermore, it has been suggested that the anti-Ro/SSA autoantibodies, might be correlated to the salivary sodium and chloride concentration (37, 39). However, no clear consensus about this has been reached thus far (37, 39).

Considering, both of these ions are increased in SjD patients when compared to various sicca patient groups, assessment of the concentrations of these ions could potentially be a good candidate to screen for SjD. However, chloride would probably be more relevant when trying to distinguish SjD patients from healthy controls, since there is a greater natural variation in the final concentrations of sodium in secreted saliva than for chloride (48). Furthermore, there is also a larger mean difference for chloride concentration between SjD patients and healthy controls, than for sodium (*e.g*. for parotid saliva: MD=23.52mM *vs*. MD=14.71mM respectively).

Both calcium and phosphate could perhaps aid in distinguishing SjD patients from healthy controls or other sicca patient groups. Phosphate, could specifically be used to distinguish SjD patients from sicca patients, as shown by the articles describing the differences between these groups (23, 31, 32, 39). In addition, the indication of a decreased phosphate output could be a way to make a differentiation with healthy controls as well. Calcium however, could be more difficult to utilise since SjD patients often suffer from an increase in oral problems, in particular dental caries

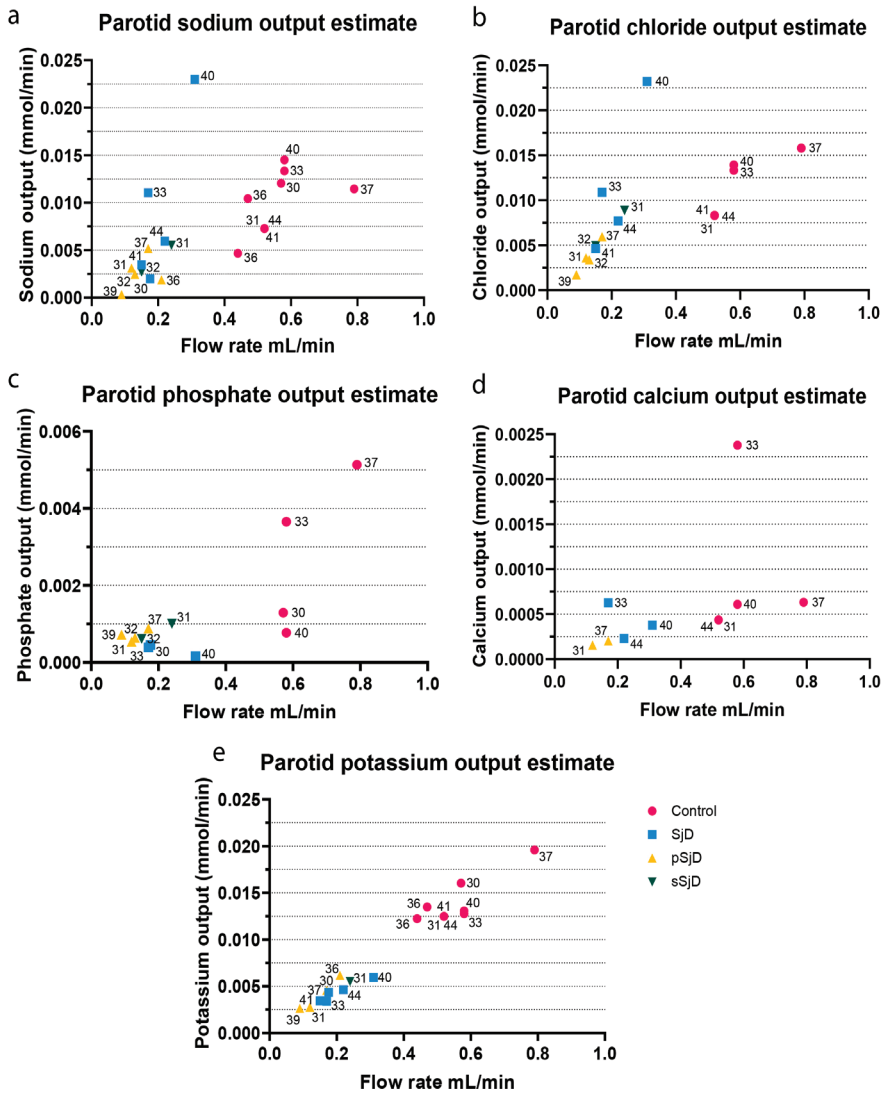

Fig. 6. Scatter plot depicting the **a**) sodium, **b**) chloride, c) phosphate, **d**) calcium and **e**) potassium output estimates against the flow rate per study for parotid saliva. Each data point corresponds to a specific patient group from one article, marked by reference number.

(50). Calcium is known to play a role in tooth demineralisation and remineralisation processes. Thus, an increase in the number of caries lesions in SjD patients could result in more free calcium in the whole saliva found in the oral cavity, which would influence calcium concentration measurements.

For potassium the results were not as consistent, because an increase in concentration was observed in UWS and a decrease in parotid saliva. It is known that potassium is mainly involved in facilitating the reabsorption of sodium in the glandular duct and partially in the process of salivary production in the glands. However, Mandel & Baurmash hypothesised that there might be another, unknown function ascribed to the secretion of potassium in the duct besides facilitating the resorption of sodium (33). Perhaps discovering whether potassium has another function in relation with saliva production and secretion, it would allow for the utilisation of this ion in the screening for SjD.

No significant differences could be attributed to the concentration of magnesium between SjD patients and healthy controls. Only one article explored these differences, so it might have to be further assessed (34). Especially since it was suggested that there are still some discrepancies in the methods for measuring salivary magnesium concentration (51).

The results for nitrite were conflicting between the studies. Where Benchabane *et al*. found an increase in nitrite concentrations, Xia *et al*. found a decrease (29, 45). Discrepancies might be attributed to differences in which SjD patients were assessed, research question and measurement techniques used. The nitrate measurements as presented by Xia *et al*. might therefore also have to be reconfirmed. These discrepancies are too great to determine whether nitrite or nitrate could be used as a marker for SjD, despite potentially promising significant differences, also when compared to sialosis patients.

The large variability in salivary ion concentrations is depended on the type of saliva tested. SWS is relatively easy to obtain, especially considering the low unstimulated flow rate of SjD patients. However, SWS has shown a greater variation within and between studies for every ion included. Often leading to no significant differences, even if they were present in other salivary types. Furthermore, depending on which ion is analysed, it might not be necessary to distinguish between the different types of SjD. Deciding which type of saliva (UWS, SM/SL or parotid saliva) to use for ion measurements might differ based on the ion of interest and the goal of the experiment. However, considering that UWS is the easiest to obtain, we conclude that it is the best choice to determine the salivary ion concentrations.

A general limitation of this study, is the limited number of articles describing the same type of SjD subgroups (pSjD, sSjD or SjD) and type of saliva (UWS, SWS, SM/SL or parotid saliva). Utilising a subgroup meta-analysis, mostly overcame this limitation. However, the use of different diagnostic/classification criteria for SjD between studies, could still have led to discrepancies in the meta-analyses. The quality of reporting of data in some articles was insufficient and thus also a limitation. Often this could be resolved by requesting additional information from the authors of the included studies. Furthermore, some studies used the same healthy control group for comparison with SjD patients, reducing the statistical power of the meta-analysis

for some of the comparisons. Lastly, the wide variety of sicca patients reported made it more difficult to draw concrete conclusion when comparing them to SjD patients.

Next it would be valuable to unravel if the increased salivary ion output in SjD is correlated to the FR. Furthermore, for salivary ions to be utilised for the early detection of SjD, the patients' disease duration needs to be considered. To investigate this, the definition of disease duration first has to be standardised in order to prevent misinterpretation of the data (23, 32, 36-38). Lastly, considering that anti-Ro/SSA autoantibodies are well-established biomarkers for SjD, resolving the current conflicting report on the correlation of anti-Ro/SSA autoantibodies with salivary ions would be a valuable insight (37, 39, 52).

To summarise, there are some differences in salivary ion levels, based on the type of saliva. SWS seemed to be the least reliable for diagnosing SjD, because it showed more variation. The other types of saliva may be more applicable, to screen for the presence of SjD in particular. Special interest is going out to UWS for salivary ion measurements. Meta-analyses suggested that chloride is the most promising ion for SjD screening. SjD patients depicted higher chloride concentration, exceeding natural deviations observed for healthy individuals at the same flow rate. By combining chloride with other ions, such as sodium, SjD patients can be better distinguished from healthy controls. However, SjD patients may be more difficult to differentiate from sicca patients. Adding phosphate might increase the ability to make this distinguishment. It is advised to conduct more research to increase the diagnostic power with ions alone or in combination with other biomarkers (*e.g.* proteins) to separate SjD from other sicca patients.

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