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Breath acetone concentration: too heterogeneous to constitute a diagnosis or prognosis biomarker in heart failure? A systematic review and meta-analysis

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Abstract

Introduction. Exhaled breath acetone (ExA) has been investigated as a biomarker for heart failure (HF). Yet, barriers to its use in the clinical field have not been identified. The aim of this systematic review and meta-analysis was to assess the ExA heterogeneity and factors of variability in healthy controls (HC), to identify its relations with HF diagnosis and prognostic factors and to assess its diagnosis and prognosis accuracy in HF patients. **Methods.** A systematic search was conducted in PUBMED and Web of Science database. All studies with HC and HF patients with a measured ExA were included and studies providing ExA's diagnosis and prognosis accuracy were identified. **Results.** Out of 971 identified studies, 18 studies involving 833 HC and 1009 HF patients were included in the meta-analysis. In HC, ExA showed an important heterogeneity ($I^2 = 99\%$). Variability factors were fasting state, sampling type and analytical method. The mean ExA was 1.89 times higher in HF patients vs. HC (782 [531–1032] vs. 413 [347–478] ppbv; $p < 0.001$). One study showed excellent diagnosis accuracy, and one showed a good prognosis value. ExA correlated with New York Heart Association (NYHA) dyspnea ($p < 0.001$) and plasma brain natriuretic peptide ($p < 0.001$). Studies showed a poor definition and reporting of included subjects. **Discussion.** Despite the between-study heterogeneity in HC, the evidence of an excellent diagnosis and prognosis value of ExA in HF from single studies can be extended to clinical populations worldwide. Factors of variability (ExA procedure and breath sampling) could further improve the diagnosis and prognosis values of this biomarker in HF patients.

1. Introduction

The World Health Organization has defined cardiovascular diseases as the first cause of death in the world [1]. Cardiovascular diseases lead to heart failure (HF) which is the main cause of hospitalization in United States, Europe and Japan. Diagnostic, prognostic and monitoring during follow-up of HF patients are based on biomarkers that can require invasive procedures (plasma brain natriuretic peptide (BNP), cardiac catheterization, coronary angiography) that can be expensive or difficult to have access to (cardiac echocardiography) [1, 2]. In order to make a more accurate diagnosis,

to monitor patients during chronic/acute phase and to identify patients with the highest risk of death/ complications/hospitalization, it is of great interest to obtain new diagnostic, prognostic or monitoring biomarkers in HF patients [3]. Methods based on the analysis of exhaled breath have allowed the identification of many biomarkers of the body metabolism through non-invasive methods (<https://hbdb.cmdm.tw/>) [4] and some of them have been validated and approved by regulating authorities for a clinical use [5]. Specifically, exhaled breath acetone (ExA) has been investigated in different diseases (cardiovascular diseases [6], diabetes [7, 8], COPD [9], etc). In HF, the ketone body utilization in the

myocardium increases together with the whole body ketogenesis [10]. Thus, exhaled acetone concentration could discriminate HF patients from healthy subjects [6], increases with the HF severity [11] and predict the patient's prognosis [12]. Thus, according to the current definition of the FDA-NIH Biomarker Working Group, the ExA could be considered as a biomarker for the diagnostic, prognostic and monitoring of HF patients [13].

However, despite these positive results in clinical studies [11], international scientific societies in cardiology do not recommend the use of ExA for clinical practice [3]. The barriers to the implementation of the ExA could be the lack of large scale diagnosis and prognosis evidence, because of the lack of multi-centric studies in HF. Another hypothesis is the lack of reference value for ExA, despite considerable measurement performed worldwide in healthy subjects. One last barrier to the translation to the clinical field could be the variability of the breath acetone concentration. If gas chromatography (GC) has become the reference method for gas detection [7, 14], new methods (ion-molecule reaction mass spectrometry (IMR-MS), selected ion flow-tube mass spectrometry (SIFT-MS), and proton-transfer-reaction mass spectrometry (PTR-MS)) are currently available to measure the exhaled acetone. In addition, experimental conditions (high temperature, massive machines, etc.), physiological factors (population, age, sex, weight, food consumption, etc), as well as other sampling factors may influence the measurements [14–16]. These multiple variability factors increase the heterogeneity of the ExA in healthy humans, and would require standardization for the clinical practice. Altogether, despite a large increase of publications since 2006 [14], researchers, engineers and physicians working on ExA have arrived at a crossroad where barriers to the development of this innovative biomarker have not been clearly identified.

Performing a systematic review is a relevant approach to establish whether findings are consistent and can be generalized [17]. It allows the identification of factors of variability as well as normal values of a biomarker [18–20]. However, previous systematic reviews in ExA did not include HF patient studies, and did not perform a quantitative meta-analysis of the data [7, 8, 21, 22]. Therefore, we aimed to systematically review the ExA concentrations from studies in healthy controls (HC) and HF patients and to meta-analyze them in order: (a) to quantify the heterogeneity of the ExA between published studies and to determine its normal range; (b) to identify factors of ExA variability and relations with HF diagnosis and prognostic factors (New York Heart Association (NYHA) dyspnea, left ventricular ejection fraction (LVEF) and plasma BNP); (c) to provide pooled estimate of its diagnosis accuracy and prognosis performance.

2. Methods

2.1. Protocol and registration

This systematic review and meta-analysis was performed according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [23]. The protocol of our study is registered in the International Prospective Register of Systematic Reviews (PROSPERO ID: CRD42020192672). The review team was composed of a cardiologist, a physiologist, a methodologist/statistician, from a university hospital, and sensor researchers.

2.2. Literature search

The literature research was conducted in English on PubMed and Web of Science to include papers published between 1991 and 2020. The collection work took place in May 2020. Additional published studies were added after analysis of reviews on the topic and on the basis of references in the articles we initially retrieved. Unpublished sources were not included. Medline Subject Headings (MeSH) database of Medline was used to define the exact keywords. A combination of the following MeSH terms was used: 'Acetone AND (Breath OR Exhaled OR Air OR Exhaled breath OR Airway) AND (Adults OR Patients OR Healthy volunteers OR Control groups)'. To minimize information bias, the study titles and abstracts were screened by three authors (D A, A V, F G) and the full texts of the original articles of potentially eligible studies were then retrieved to obtain complete details for inclusion. Study selection was on the basis of agreement of two authors and, in cases of disagreement, the consensus of three authors (D A, A V, F G) was sought.

2.3. Study selection

Studies were included in the systematic review and meta-analysis if they met the inclusion criteria (supplementary table 1 (available online at stacks.iop.org/JBR/16/016001/mmedia)). We included every type of study reporting a measured concentration of acetone in the exhaled breath of subjects defined as adult HF patients or adult healthy subjects. The study type had to be clinical. Thus, a minimum of 15 subjects should be included in order to exclude laboratory validation studies. The design had to be comparative with acetone concentrations compared between healthy subjects and patients. Every type of analytic method was included in the study and the methodology had to be detailed. HF patients were defined according to the conclusion of a medical examination and echocardiography (showing LVEF < 50%) performed by a medical doctor. If information regarding the inclusion criteria were lacking in the study full text, the authors were contacted (D A). If no answer was obtained the study was excluded. Duplicates were identified and removed from the analysis. In addition, we further

identified studies eligible for the diagnostic and prognostic accuracy assessment, by adding the following criteria: (a) an original comparative study including well defined populations of HF patients and HC and (b) providing receiver operating characteristic (ROC) curve analysis parameters (for a diagnostic marker) or providing survival analysis parameters (for a prognostic marker).

2.4. Data extraction

Studies providing charts only for acetone concentrations were included in the qualitative synthesis but not in the meta-analysis. If the breath sampling occurred in different conditions (sitting or lying down, during a workout, etc), only concentrations assessed at baseline was extracted. Data were extracted blindly and independently by three independent authors using a standardized form (see supplementary materials online) (D A, F G, C H). When discrepancy occurred, the final data record was based on consensus (F G, C H). From the eligible articles, we extracted the acetone concentrations in volumic part per billion (ppbv). To standardize the unit, acetone concentrations in mol l⁻¹ or g l⁻¹, were converted in ppbv, using the acetone molar volume and molecular molar mass (24.79 l mol⁻¹ and 58 g mol⁻¹ at $P = 1$ bar, $T = 25$ °C, respectively) and the following simple calculations:

$$[\text{Acetone}] (\text{ppbv}) = ([\text{Acetone}] (\text{g l}^{-1}) \times 24.79 \div 58) \times (10^9)$$

$$[\text{Acetone}] (\text{ppbv}) = ([\text{Acetone}] (\text{mol l}^{-1}) \times 24.79) \times (10^9).$$

We also extracted age, sex ratio, body mass index, disease status, NYHA symptom classification of dyspnea, clinical state (stable/acute HF), LVEF (%), measurement method, sampling method, fasting state, smoking status and ventilatory procedure (breath-hold, control of expiratory flow, alveolar washout) because all these factors have been shown to modify the ExA concentration (supplementary table 2). When acetone concentrations showed discrepancies, authors were contacted by email to confirm the data. If no answer was obtained, the study was excluded from the meta-analysis. In addition, for diagnostic and prognostic accuracy studies, we further extracted ExA cutoffs, area under curve (AUC) values and its confidence interval (CI), and the hazard ratio (HR), and its variance and/or standard error of the mean (SEM) and/or CI.

2.5. Study quality assessment

As the selected studies were mostly observational with a cross-sectional design the study quality was assessed using the journal's impact factor and discipline, the study's aim (clinical/sensor development), the

'selection' items of the Newcastle Ottawa Scale (NOS) [24], the procedure of volunteer selection (medical examination), the inclusion/exclusion criteria, the description of the study's population (HF etiology, medications, and other characteristics). Finally, the risk of bias (selective reporting, etc) was assessed.

2.6. Statistical analysis

Quantitative data are presented as means \pm standard deviation (SD) and means [95% confidence interval]. Median, min-max, SEM and interquartile range were converted according to Shoman *et al* [20]. If there were subgroups with different sample size (n_1 , n_2 , etc), data were pooled into a single group (sample size: n_{tot}) and the SD was calculated according to the following formula:

$$SD = \sqrt{\left(\left(\frac{n_1}{n_{\text{tot}}}\right)^2 \times SD_1^2\right) + \left(\left(\frac{n_2}{n_{\text{tot}}}\right) \times SD_2^2\right) + \dots}$$

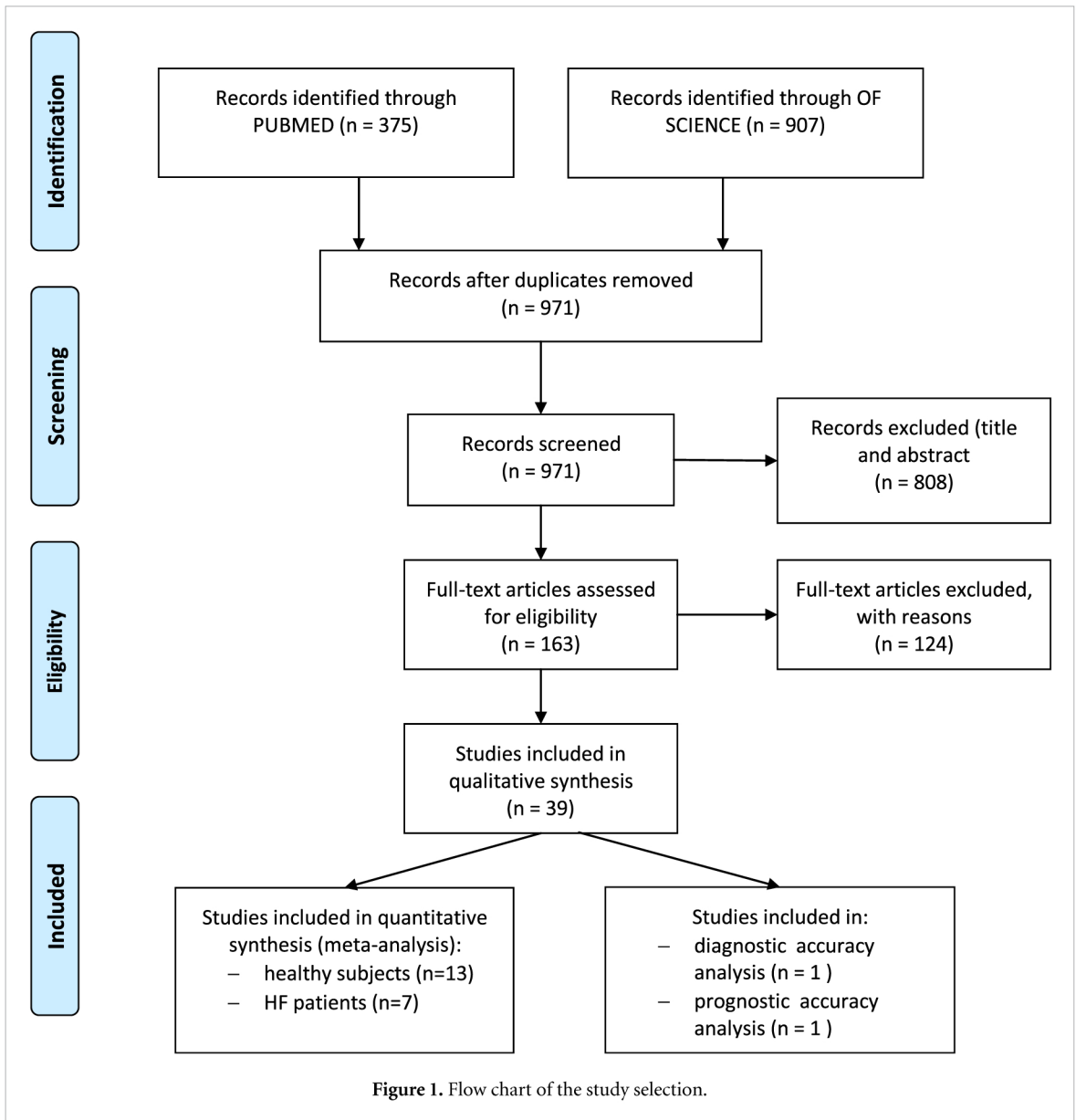
Forest plots were used to graphically evaluate both the variability (i.e. SD) of the data and the weight of each study, according to its population size and variability. Statistical heterogeneity was quantified by the Q-Cochrane heterogeneity test (Q statistic with degree of freedom (df)) and the I^2 statistic. In case of heterogeneity, a random effect model was performed. Differences between subgroups were tested using the t -test. When there were sources of heterogeneity, meta-regressions were performed to test whether factors like BNP and LVEF (%) had an impact on breath acetone concentrations. Analyses were performed using R 4.0.3 software (www.r-project.org). P values were considered significant if $\alpha < 5\%$. Yet, to adjust for multiple pairwise comparisons, a Bonferroni correction was applied for α .

3. Results

3.1. Study selection

The search equation performed in PubMed and Web of Science (supplementary table 3) retrieved 375 and 907 articles, respectively. Figure 1 details the flow of studies included in the review.

First, the study eligibility evaluation was performed on these 971 papers according to the title and abstract. The details of the reason of exclusion are given in the supplementary table 4. Thirty-nine studies were included for the qualitative synthesis and a final library of 18 studies (13 in healthy subjects and seven in HF patients) were included in the meta-analysis. Two studies have directly compared HF patients to HC and were included in the diagnostic and prognostic accuracy analysis [25, 26]. Seven studies have compared ExA concentration in HC to other patients (diabetes [27, 28], cancer patients [29, 30],



respiratory [31, 32], or liver disease [33]). Nine studies were not comparative [6, 11, 34–39]. These studies involved 1842 subjects (883 healthy subjects and 1009 HF patients), with a mean sex ratio of 0.56 and mean age varying from 29.3 ± 11.9 [28] to 77.0 ± 24.0 years [40] in studies (table 1). All studies included in the meta-analysis were cross-sectional. Subjects were recruited worldwide, with $n = 659$ (35.8%) recruited in Europe, $n = 254$ (13.8%) in Asia, $n = 125$ (6.8%) in North America and $n = 804$ (43.6%) in South America (all from Brazil). In the 14 studies reporting the subject's smoking status, 23% of the subjects were current smokers and 77% were non-smokers.

3.2. Heterogeneity of the exhaled breath acetone concentration in healthy subjects

Forest plots showed an important heterogeneity of breath acetone concentrations between studies in healthy subjects, with $I^2 = 99\%$ ($p < 0.01$). Random

effects model showed pooled values = 413 (347–478) ppbv in healthy subjects, with mean values ranges from 167 to 720 ppbv in the included studies (figure 2). Given the important between-study heterogeneity in healthy subjects, it was not possible to provide a normal range of ExA concentrations.

Forest plot reports ExA concentrations (raw means (MRAW) and 95% confidence interval (CI), in ppbv) in healthy subjects for the 13 studies included in the systematic review.

3.3. Determinants of the exhaled breath acetone concentration heterogeneity

Pairwise comparison between ExA in Europe, Asia, and America versus rest of the world in healthy subjects and HF patients did not reveal any significant effect of the population on the ExA value ($p > \alpha$ corrected = 0.002). In HF patients, studies in Brazilian HF patients reported ExA values that were not statistically different for ExA obtained in HF patients in the

Table 1. Exhaled breath acetone concentrations in the included studies.

Study	Sample size	Control subjects		M/F	Age	Fasting (1/0)	Acute HF (1/0)	NYHA Class	% LVEF	GC (1/0)	Acetone concentrations
		(C)/HF patients (HF)	(HF)								
Marcondes-Braga <i>et al</i> [11]	695	HF	HF	470/225	55.4 ± 12.2	0	1	I = 130	32 ± 8.6%	0	256.4 (128.2–512.8)
								II = 433			
								III = 127			
								IV = 5			
Yokokawa <i>et al</i> [40]	8	HF	HF	2/6	71 ± 8	1	0	I = 3	39 ± 15%	1	730 (600–920)
								II = 4			
								III = 1			
								IV = 0			
Biagini <i>et al</i> [39]	40	HF	HF	8/11	77 ± 24	1	1	I = 0	42 ± 18%	1	2400 (1620–6080)
								II = 0			
								III = 15			
								IV = 5			
Yokokawa <i>et al</i> [38]	102	HF	HF	57/45	63.6 ± 15.8	1	1	I = 4	50.1 ± 15.3%	0	530 (330–880)
								II = 12			
								III = 17			
								IV = 7			
Dolch <i>et al</i> [31]	17	C	C	10/7	26–55	0	—	I = 10	—	0	631 (520–784)
								II = 75			
								III = 17			
								IV = 0			
Li <i>et al</i> [27]	56	C	C	24/22	21–71	1	—	II = 17	—	1	720 ± 374
								III = 15			
								IV = 5			
								I = 4			
Zhou <i>et al</i> [28]	44	C	C	25/19	NK	0	—	I = 10	—	1	488 ± 166
								II = 7			
								III = 17			
								IV = 7			
Ma <i>et al</i> [30]	25	C	C	16/9	NK	0	—	II = 7	—	1	247
								III = 17			
								IV = 0			
								I = 4			
Samara <i>et al</i> [6]	25	HF	HF	NK	NK	1	0	I = 10	27 ± 13%	0	811 (256–1974)
								II = 75			
								III = 17			
								IV = 0			
Marcondes Braga <i>et al</i> [25]	30	HF	HF	19/11	42–56.5	0	0	I + II = 20	25.0 (20.0–31.5)	1	521.4 (290.6–936.0)
								III = 10			
								IV = 0			
								I = 0			

(Continued.)

Table 1. (Continued.)

Study	Sample size	Control subjects (C)/HF patients (HF)	M/F	Age	Fasting (1/0)	Acute HF (1/0)	NYHA Class	% LVEF	GC (1/0)	Acetone concentrations
	59	HF	36/23	46–63	0	1	I + II = 0 III = 29 IV = 30	23.0 (1.0–29.0)	1	3333 (1538.6–6496.6)
Ulanowska <i>et al</i> [29]	20	C	9/11	43.3–55	0	—	—	—	1	166.6 (128.2–299.2)
Schwarz <i>et al</i> [34]	143	C	18/68	21–56	0	—	—	—	1	225.7 [41.6–753.4]
	215	C	95/148	M: 52 ± 17 F: 51 ± 19 NK	0	—	—	—	0	628 ± 1.56
van den Velde <i>et al</i> [33]	50	C	21/29	NK	0	—	—	—	1	212.3 (144.6–32.9)
Turner <i>et al</i> [35]	30	C	19/11	45.8 ± 9.3	0	—	—	—	0	531.3 ± 205.3
Barker <i>et al</i> [32]	20	C	18/22	8–29	0	—	—	—	1	469 [383–551]
Euler <i>et al</i> [36]	100	C	62/38	S: 44.7 ± 1.6 NS:	0	—	—	—	1	364.4 ± 54
				48.0 ± 2.3						
Kupari <i>et al</i> [26]	31	HF	15/16	56 ± 16	1	0	II = 15 III + IV = 16	38 ± 18%	1	2007.9
	24	C	14/10	41 ± 10	1	—	—	—	1	471 (R: 198.3–1165.1)
Wang <i>et al</i> [37]	39	C	NK	20–58	0	—	—	—	1	305.6 ± 232.9

M/F: male/female population; 1/0: yes/no; NK: not known; R: range.

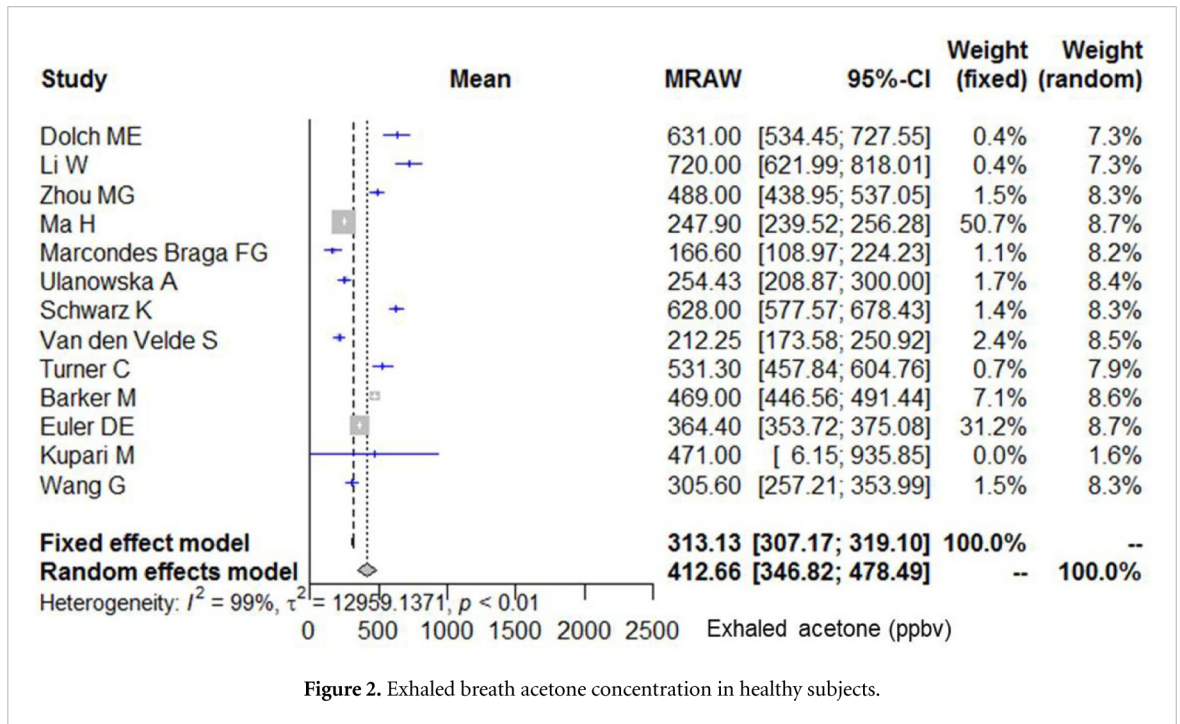


Figure 2. Exhaled breath acetone concentration in healthy subjects.

rest of the world. (907 (369–1445) vs. 787 (502–1072) ppbv; $p = 0.70$). Neither the age nor the sex ($p = 0.185$ and $p = 0.40$, respectively; supplementary figure 1) of the subjects was significantly correlated with the concentration.

In HC and HF patients, studies that have included more than 25% of smokers showed ExA concentrations that are not significantly different from studies that have included less than 25% smokers (290 (198–381) vs. 388 (197–580) ppbv; $p = 0.36$). In all studies, fasting condition was imposed to subjects, with fasts ranging from at least 8 h [2, 22, 23, 34, 36, 37], or between 2 h and 30 min [11, 39]. In healthy subjects, fasting condition was imposed in only two studies (8 and 12 h) [26, 27]. We observed that fasting acetone concentrations were higher than uncontrolled diet concentrations (703 (581–825) ppbv vs. 387 (320–454) ppbv; $p < 0.001$).

Regarding the method, the acetone concentration was obtained by GC and GC-MS (mass spectrometry) in 10 healthy subjects studies, representing 571 healthy subjects (mean age: 40.3 years old, sex ratio (M/F): 0.46) [25–28, 30, 32, 33, 36, 37]. The three other studies used IMR-MS [31], SIFT-MS [35] and PTR-MS [34]. Comparing the methods, GC and GC-MS studies displayed lower values than other type of sensors 355 (288–422) ppbv vs. 597 (532–662) ppbv; $p < 0.001$). Only 1/10 study showed a mean GC concentration [27] above the lowest mean concentration obtained with another type of sensor [35]. However, even with data restricted to this method, the heterogeneity remained important ($I^2 = 99\%$; $p < 0.01$; figure 3).

Forest plot reports the ExA concentrations (raw means (MRAW) and 95% confidence interval (CI), in

ppbv) in healthy subjects with GC and/or GC-MS for the 10 studies included in the systematic review.

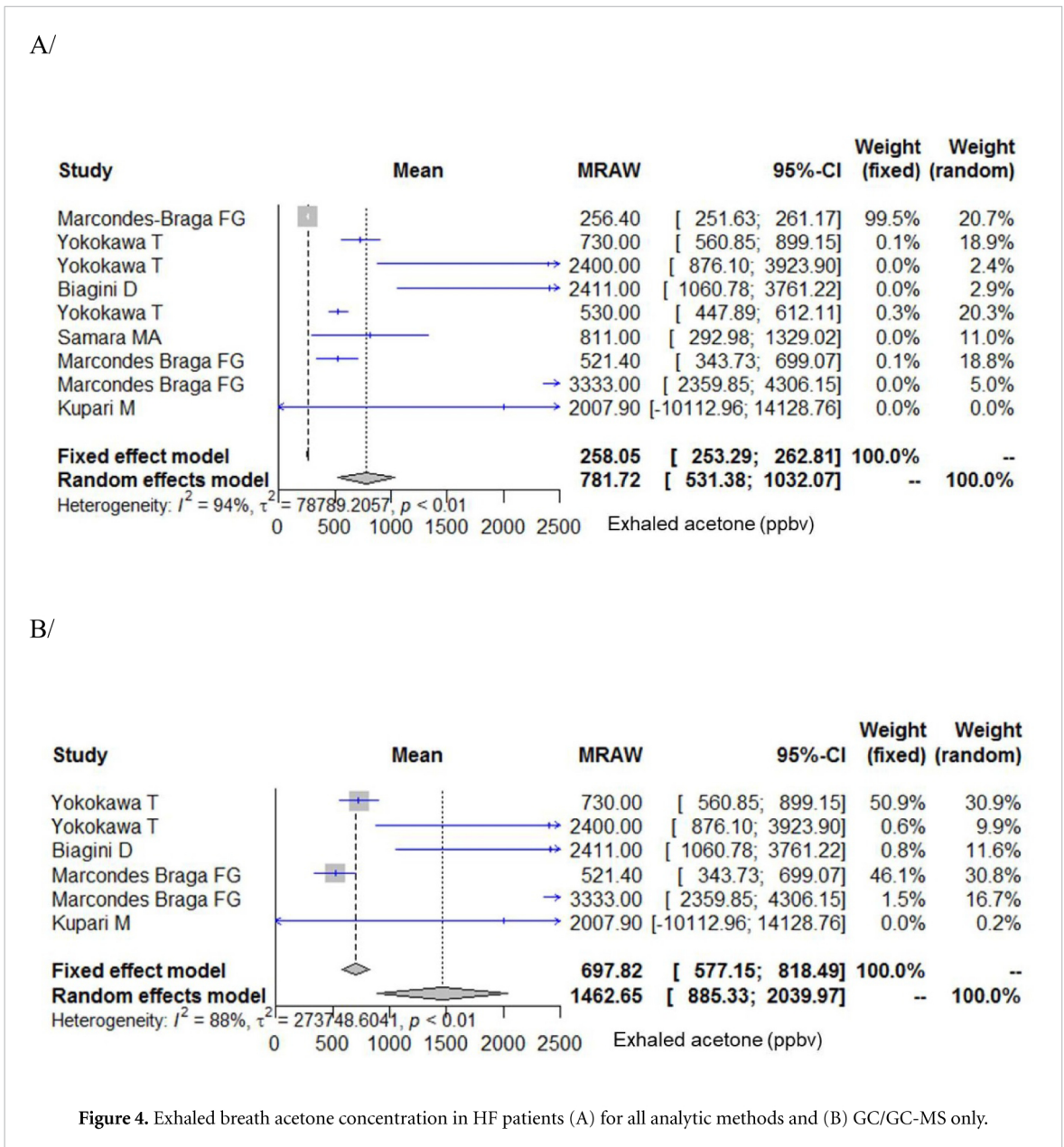
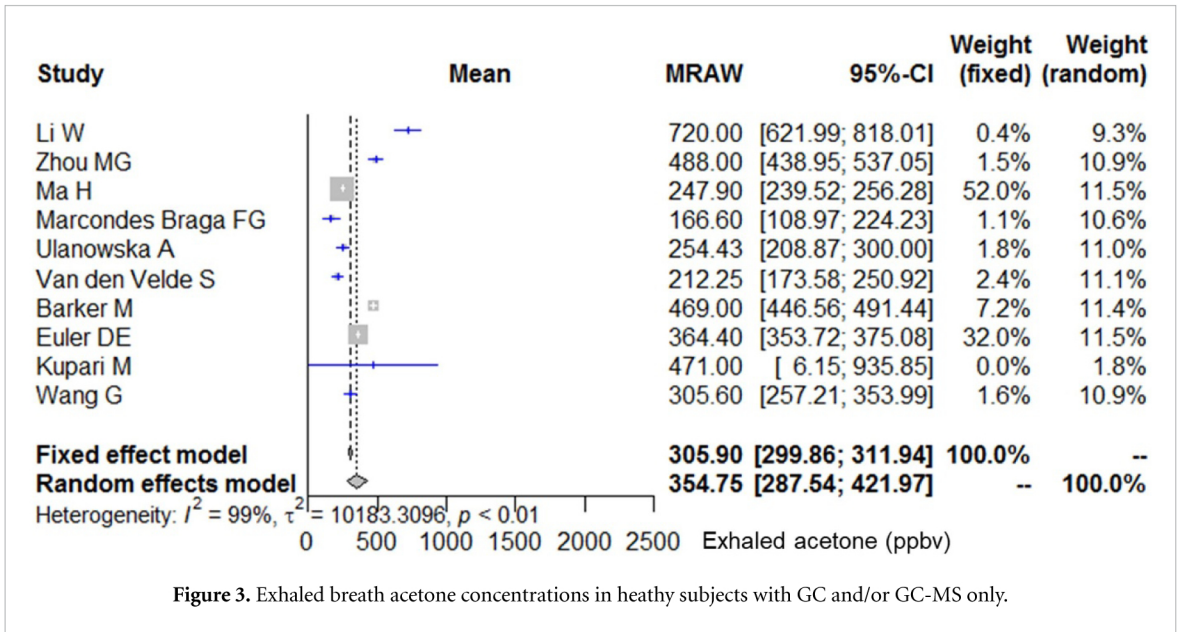
Online measurement systems (IMR-MS, SIFT-MS and PTR-MS) showed higher values than offline systems that used bags or other containers to store the exhaled breath before analysis (576 (479–623) ppbv vs. 382 (313–452) ppbv; $p < 0.001$). Alveolar sampled or end-tidal acetone concentrations did not differ significantly from total breath sampling concentrations (377 (306–448) ppbv vs. 488 (249–727) ppbv; $p = 0.38$).

3.4. Relations with HF diagnosis and prognostic factors

HF patients represented 7/20 studies and 54.8% ($n = 1009$) of the total population of the meta-analysis. ExA concentrations were 1.89 times higher in HF patients than in healthy subjects, for all type of analytical methods (782 (531–1032) vs. 413 (347–478); $p < 0.001$) and 4.12 times higher for GC and GC-MS only (1463 (885–2040) vs. 355 (288–312) ppbv $p < 0.001$). The heterogeneity was still important between studies in HF patients (figure 4), with an $I^2 = 94\%$ ($p < 0.001$) and 88% ($p < 0.001$) for all analytic methods and GC/GC-MS only, respectively.

Forest plot reports ExA concentrations (raw means (MRAW) and 95% confidence interval (CI), in ppbv) in HF patients studies included in the meta-analysis.

Exhaled acetone concentrations were higher when measurements were obtained in acute HF patients than in studies including stable HF patients (Biagini *et al.*: 2411 \pm 4357 ppbv [39]; Yokokawa *et al.*: 2400 \pm 3389 ppbv [40]; Samara *et al.*: 811 \pm 1322 ppbv [6]; Marcondes-Braga *et al.*: 3333 \pm 3814



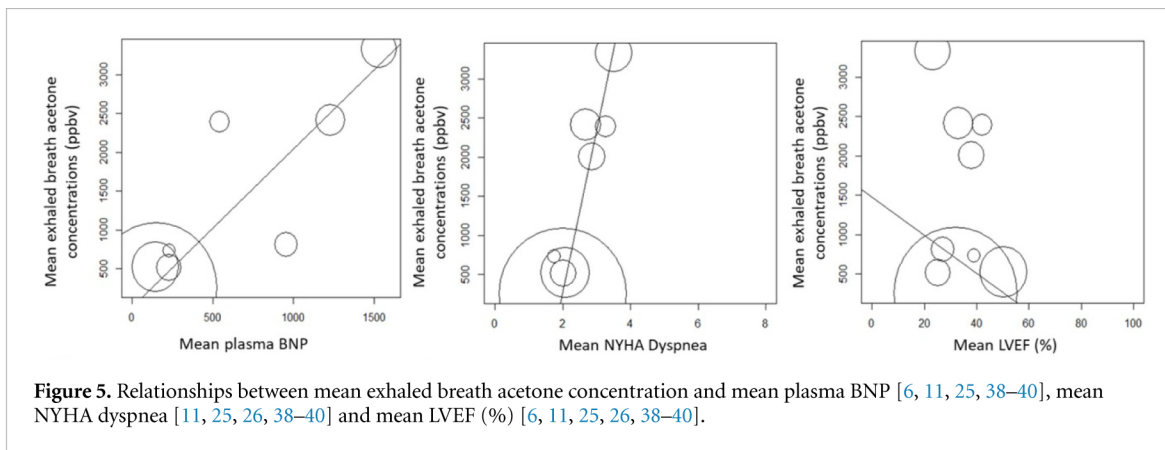


Figure 5. Relationships between mean exhaled breath acetone concentration and mean plasma BNP [6, 11, 25, 38–40], mean NYHA dyspnea [11, 25, 26, 38–40] and mean LVEF (%) [6, 11, 25, 26, 38–40].

ppbv [25]). In HF patients, the univariate analysis showed significant and positive correlations between breath acetone concentration and NYHA dyspnea ($\beta = 2051.4$; $p < 0.001$), and plasma BNP ($\beta = 2.0417$; $p < 0.001$) (figure 5). However, there was no significant correlation between breath acetone concentration and the echocardiography LVEF (%) ($\beta = -24.17$; $p = 0.648$).

Circle sizes represent group sizes. Solid lines represent the weighted linear regression lines. BNP, brain natriuretic peptide; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; ppbv, volumic part per billion. Exhaled acetone concentration = $2051.4 \times \text{NYHA} - 3810.5$; exhaled acetone concentration = $2051.4 \times \text{LVEF} + 1477.31$; exhaled acetone concentration = $2.0417 \times \text{BNP} + 0.3434$.

3.5. Diagnosis accuracy and prognosis performance of exhaled breath acetone in HF patients

In HF patients, only two studies provided diagnostic [25] or prognostic [11] accuracy parameters. For an ExA concentration cutoff = 496 ppbv, Marcondes-Braga *et al* reported an AUC = 0.94 (95% CI, 0.91–0.99), meaning that with this ExA cutoff, the sensitivity was 83%, the specificity was 100%, and the accuracy was 86% for differentiating patients with HF ($n = 89$) from healthy subjects ($n = 20$) [25]. Using another sensor, Marcondes-Braga *et al* reported that HF patients with ExA > 513 ppbv had the poorest prognosis, with an adjusted HR = 2.52 (1.24–5.13) [11].

3.6. Quality assessment of the studies

The synthesis of the quality assessment is reported in supplementary table 5. All the studies included have been published in peer-review international journals. If three studies presented analytical validation data of the sensor being developed by the authors, all of them aimed to perform a clinical validation of the sensor. Thus, studies were mostly published in journals of the medical research field (11/18, 61%), 7/18 (39%) in journals of biology, biochemistry or chemistry fields, 7/18 (39%) in journals of the engineering science, biophysics, technology fields and 1/18 (6%)

in a multidisciplinary sciences journal. Accordingly, the mean sample size for the control and HF patient groups was 88 ± 151 (64 ± 59 and 202 ± 352 in HC and HF patients, respectively), with one study having included 695 HF patients. However, in the 13 healthy subject studies, the inclusion procedure was detailed only in four of them, and exclusion criteria reported also in four studies. The poor quality of the control subjects selection is highlighted by the score of the ‘selection’ items of the NOS, with a mean value of 0.77 ± 0.60 , and reaching 2/2 in one study only [27]. Indeed, the source and the definition of the HC subjects were rarely specified. A table of characteristics of the healthy subjects was provided in only 5/13 studies. Regarding the seven HF patient studies, the NOS ‘selection’ items was 1.29 ± 0.76 , with 3/7 studies reaching the maximal value of two points. The inclusion procedure and exclusion criteria were lacking in two studies. In five of them, etiology and treatment of the HF have been reported, and a table of characteristics was present in six of them. However, these information revealed a limited representativity of the HF patients included in these studies. Indeed, a significant prevalence of Chagas disease was found in two studies, B-blockers was an exclusion criteria in two studies [25, 26], and two studies revealed a large gap of patients between HF patients screened and included [25, 38].

4. Discussion

While several reviews on ExA have been published in healthy and diseased patients, it is the first systematic review providing quantitative and generalizable data regarding ExA concentrations in healthy adults worldwide. Standardization factors (fasting), methodological issues (sensors, sampling and analytical methods), and HC definition altered the between-study homogeneity. Conversely, the pooled ExA concentrations in HF patients was 1.89 times higher than in healthy subjects and correlated with the NYHA dyspnea scale and plasma BNP, highlighting its good diagnostic and prognostic accuracy in single studies.

4.1. Determinants of variability in healthy subjects

In healthy subjects, the I^2 was 99% ($p < 0.001$), meaning that the heterogeneity was important. This heterogeneity may reveal physiological factors of variability of the exhaled acetone. The role of the age on ExA concentration has been found in previous studies. Schwarz *et al* found a significant and positive correlation between the exhaled acetone concentrations and the age [34] and younger subjects (17–18 years) had lower ExA concentrations than adults (20–60 years) [41, 42]. Gender may also impact ExA concentrations, as a 24% difference in the exhaled acetone concentrations has been reported between healthy males and healthy females [35]. However, our regression analysis did not reveal the contribution of the age and sex ratios to the ExA concentration heterogeneity, probably because of a limited distribution of the age and sex ratios in the included studies (supplementary figure 1). The tobacco smoking could also impact the exhaled acetone concentrations. However, in line with the results, Euler *et al* showed no significant difference between smokers and nonsmoking healthy subjects, and breath samples obtained before smoking and at 5, 15 and 60 min after smoking did not show any significant changes in acetone concentration [36].

Acetone (C_3H_6O) is a ketone body mainly (but not solely) produced in the liver and released during the fatty acids metabolism and are used as energy substrates. At high plasma acetone concentrations, approximately 80% of the acetone production rate was attributed to exhaled breath. Thus, in diabetic patients, the exhaled breath was positively correlated with the plasma acetone concentrations during ketoacidosis [43]. In addition, exercise, sleep and diet also alter exhaled acetone concentrations [44, 45]. In particular, fasting increased breath acetone while high carbohydrate feeding decreased or maintained breath acetone at low concentration [46, 47]. In our meta-analysis, the comparison of the two studies with a ≥ 8 h fast ($n = 80$ [26, 27]) versus other studies with a shorter or unstandardized fast ($n = 753$) is in line with the effect of the fasting on the ExA in previously published studies. Thus, the diet should be controlled by standardizing the meals or the fasting duration before the measurement, in order to reduce the acetone variability.

4.2. Analytical method, sensor and sampling

Mass spectrometry is used to identify and quantify a compound according to its chemical mass. In the selected studies, MS is combined with GC [25–28, 30, 32, 33, 36, 37], IMR-MS [31], PTR-MS [34] and SIFT-MS [6, 35]. These methods are used to separate the different compounds of a mixture before their identification by MS. Considering the very low concentration of acetone (ppbv) in the exhaled breath, all of these techniques were adapted for the requirement. Indeed, the preclinical validation data have shown

low and comparable limit of detection (pptv). While GC is seen as a ‘gold standard’ in analysis of volatile compounds in breath research [48, 49], only few studies have compared new techniques like PTR-MS [50, 51] or SIFT-MS [52, 53] with GC-MS. Real time analysis is limited with GC in contrast with PTR-MS, SIFT-MS, IMR-MS methods. Thus, the evaluation of different pulmonary compartments would be more appropriate with these new methods versus GC for the quantification of exhaled breath gases [54]. However, different authors have recently pointed that PTR-MS, SIFT-MS, IMR-MS may not be able to separate molecules with the same weight, as it is done by GC-MS [7, 49, 55]. Thus, when the gaseous mixture is as complex—as in the exhaled breath—GC-MS would provide a more accurate identification and quantification of the gas concentrations [49, 56]. Nevertheless, the between study heterogeneity was not reduced in GC-MS vs. all studies included in our meta-analysis. In addition, acetone is the dominant signal at the corresponding mass-to-charge ratio (m/z), with other compounds unlikely to have any significant contribution in most cases. Wang *et al* have pointed factors that hamper the stability of GC-MS measurements [57] and could contribute to the important between-study heterogeneity remaining in GC-MS studies.

While acetone is mostly eliminated through *in vivo* metabolism, lungs and urine elimination account for 2%–30% of its endogenous production [58]. The concentrations of water-soluble, blood-borne substances in exhaled air are dependent on the breathing patterns and on the airways and the alveolar/systemic concentrations of the compound [16]. The impact of the sampling on the exhaled breath concentrations heterogeneity should be discussed in the light of the acetone exchanges in all lung compartments. Experimental data and theoretical predictions [59] suggest that acetone does not come predominantly from alveoli, but also from the airways of the lungs [1]. In 9/13 of the control subject studies of the meta-analysis, the first portion of the breath (‘dead space air’) was discarded and the end-tidal concentration was measured or sampled, assuming that it reflected the alveolar concentration. However, acetone partial pressure in end-tidal expiratory samples reached only $\sim 80\%$ of the acetone alveolar partial pressure [1]. Thus, an underestimation of the alveolar concentration would be $\sim 20\%$ sampling the total vital capacity, and $\sim 30\%$ sampling the tidal volume. If this larger underestimation in tidal volume vs. alveolar sampling was not found in our systematic review, the contribution of the airway exchange as well as the flow rate to the acetone concentrations contribute to increase the intra- and inter-study heterogeneity.

4.3. Quality of the reporting, quality assessment

Because of the inclusion criteria (study type, minimum sample size) results have been mostly

published in clinical research field journals, with some of them being top-ranking journals in their field. The sample sizes appeared adequate to answer the question of the study, with $n = 64 \pm 59$ HC and $n = 202 \pm 352$ HF patients included. However, the standards for the population definition and reporting were generally not met. The source, definition, inclusion criteria, characteristics of the healthy subjects and patients were not found in most articles. This issue may explain a part of the between study heterogeneity in our meta-analysis. In addition, in HF patients, there was a discrepancy between studies regarding the inclusion criteria (B-blocker [11, 25, 26]), and the representativity of the sample [25, 38], which may even alter the between study heterogeneity.

4.4. Reference values for exhaled breath acetone concentration: a step forward

In our study, the pooled estimates for ExA in HC were 413 [347–478] ppbv for all sensors and 355 [288–422] ppbv for GC-MS sensors only. Although these pooled estimates cannot be considered as reference values given the important between study heterogeneity ($I^2 = 99\%$), these values constitute a step forward to the identification of a normal range for ExA concentration. Indeed, previous reviews stated that ‘exhaled breath contains acetone that varies from 300 to 900 ppb in healthy people’ [7, 8, 60]. Unfortunately, this ‘normal range’ was referenced on two studies with a limited population sample. A first GC-MS study reported values lower than 800 ppbv in 15 subjects [61] and a second SIFT-MS-based study reported values ranging between 293 and 870 ppbv in only five subjects [62]. Wang *et al* reported mean values ranging from 390 to 1090 ppbv on the basis of 12 HC studies [63]. However, this review was not systematic and no pooled mean and 95% CI were provided [63]. Thus, it is logical that the 95% CI in our meta-analysis—in spite of the important study heterogeneity—is way narrower than the range proposed in these previous reviews. Consequently, our pooled mean ExA concentration questions previous published studies reporting acetone concentration as high as 1200 ppbv in healthy subjects [64, 65], or as low as 100–200 ppbv in HF patients [11]. Therefore, the acetone concentration reported in our meta-analysis as well as its determinants constitute a relevant tool for researchers and engineers working in the development of acetone sensors. In addition, it constitutes the basis for a large clinical study to standardize the procedure and implement reference values for ExA concentrations.

4.5. Exhaled acetone in heart failure patients: a diagnostic and prognostic biomarker

The FDA-NIH Biomarker Working Group has recently standardized the definitions of diagnostic,

prognostic monitoring and predictive biomarkers, based on their ability to improve medical decisions [13]. A diagnostic biomarker is ‘a biomarker used to detect or confirm presence of a disease or to identify individuals with a subtype of the disease’ [13]. Its performance under the defined conditions should be characterized with an adequate prevalence of the disease that the test aims to diagnose. While our systematic review retrieved 13 studies in healthy subjects and seven in HF patients, only one provided diagnosis accuracy parameters [25]. In this study, ROC analysis showed an excellent discriminative property, with an AUC > 0.90 [66], for an ExA cutoff = 496 ppbv. This diagnosis accuracy in HF was largely higher than the moderate diagnosis accuracy reported in diabetes (AUC = 0.79 (95% CI 0.75–0.82) [67]). Yet, it was not assessed in intent-to-diagnose population study, because from the 235 HF outpatients screened, 89 were finally included. Thus, the exclusion of 62% of the HF patients screened should alter the discriminative property of ExA in real-life settings [25]. Yet, versus HC, the ExA concentration were 3.13 times higher, which is close to the 1.89 times higher concentration found in the 1009 ‘real-life’ HF patients included worldwide in our meta-analysis. Moreover, we were not able to find any significant difference between ExA concentrations reported in HF patients and HC in Brazil vs. other countries. Thus, one could expect that the diagnostic performance would remain acceptable if ExA assessment was generalized to all HF patients.

A prognostic biomarker is ‘a biomarker used to identify likelihood of a clinical event, disease recurrence or progression in patients who have the disease’ [13]. Our systematic review retrieved one large ($n = 695$) yet mono-centric Brazilian study that provided prognostic accuracy assessment of ExA in HF patients [11]. The relative risk of death in patients with ExA > 513 ppbv was 2.52 higher than in patients with ExA $< \text{or} = 513$ ppbv. In this prospective *a priori* defined cohort study, drawbacks were limited and ExA assessment performed in stable outpatients with HF defined according to the Framingham criteria, regardless of the HF etiology [11]. Among the ExA quartiles, there was a significant increase in HF symptoms and plasma BNP [11], which is in line with the significant correlations observed with the NYHA dyspnea scale and plasma BNP in our systematic review of HF patients worldwide. These correlations remained significant in spite of the lack of standardization of the method and the heterogeneity in the patient’s selection. Last, in four studies, acute HF patients had the highest ExA concentrations. Altogether, our systematic review and meta-analysis extend the existing evidence for the diagnostic and prognostic performance of ExA in HF patients.

4.6. Exhaled breath acetone measurement for HF in cardiology practice

The patient's prognostic stratification and monitoring constitute major issues in HF patients [68]. Indeed, the recent European data (ESC-HF pilot study) has reported that the 12 month all-cause mortality and hospitalization rates differ greatly in hospitalized (17% and 44%, respectively) and stable/ambulatory HF patients (7% and 32%, respectively). The risk stratification remains challenging in HF [3, 69]. Currently, plasma BNP and the echocardiography remain the current 'gold standards' for the prognostic assessment. However, the plasma BNP requires a peripheral blood sample and is quite expensive. In contrast, echocardiography is non-invasive, but it requires a specific expertise and equipments. Thus, intense research has allowed the identification of numerous new prognostic markers in HF patients. In this context, ExA has appeared as a good candidate. Indeed, ketone utilization in the myocardium together with the body ketogenesis and plasma ketone concentration increased in proportion to the severity of the HF [10]. Yokokawa *et al* have provided an analytical validation of ExA, showing a good agreement between ExA and reference method for the HF diagnosis (i.e. the pulmonary capillary pressure) [38]. In this context, our meta-analysis provides larger evidence for the value of ExA in the HF patients risk stratification. The significant correlations between ExA, plasma BNP and mean NYHA dyspnea obtained in eight studies ($n = 978$ and 984 , respectively) worldwide and regardless of the method are in line with the single prospective monocentric study. In addition, it is non-invasive and based on a simple procedure. In acute respiratory distress, distinguishing between a cardiac and pulmonary origin is challenging and additional investigations are recommended [3]. Our meta-analysis studies showed higher ExA concentrations in studies including acute HF patients vs. stable HF patients, in line with the excellent discriminative accuracy reported in one previous study. Because ExA measurement could provide immediate results at the bedside, it could help initiating an early and appropriate therapy and avoid a patient's hospitalization. However, cross-sensitivity with diabetes can be expected, because the diagnosis accuracy has been assessed in non-diabetic patients [25] and because ExA increased with the plasma acetone concentration during diabetic ketoacidosis [43]. Thus, a potential limitation for the use of ExA in clinical setting is the presence of diabetes comorbidity in a HF patient. Last, a significant decrease of the ExA concentration after acute HF treatment has been reported [40] and suggests a potential monitoring value for ExA. While this remains to be tested in clinical settings, the potential of miniaturization of the ExA sensors and device opens perspective for telemonitoring and 'point-of-care' testing in HF patients [70].

In the modern era, medical decision requires biomarkers. A first strategy is to use reference values with means and limits of the normal of the biomarker. However, this requires large and correctly defined measurements in healthy populations. Our systematic review and meta-analysis approach allowed estimating ExA concentrations in 833 healthy subjects (i.e. 412 (347–478) ppbv). If it constitutes the most adequate range published to date, it cannot be considered as reference values, because of large heterogeneity between studies. Yet, our analysis points barriers that should be overcome to obtain ExA 'reference values' for a clinical use. First, ExA measurements should be standardized and at least performed after overnight fast, as for glycemia and lipid profile dosage. In addition, reference values should be obtained worldwide in well-defined and fully characterized community-based healthy subjects, using a device with a good performance (i.e. GC-MS and/or other) and considering the acetone exchanges in the airways. The second strategy for a validated biomarker is to define decision cutoffs. ExA concentration and correlations with severity markers in 1009 HF patients worldwide confirms the excellent diagnostic and prognostic accuracy reported in monocentric studies. Altogether, our study provides additional evidence that ExA constitutes a diagnostic and prognostic biomarker in HF patients. In parallel to the standardization of the procedure, the assessment of the diagnostic, prognostic and monitoring accuracy in HF patients and HC appears as the most relevant strategy to obtain ExA concentrations cutoffs for medical use.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

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