Systematic review of biomarker findings from clinical studies of electronic cigarettes and heated tobacco products

Yukio Akiyama a,1,*, Neil Sherwood b,1

a Department of Environmental Management, University of Occupational and Environmental Health, Kitakyushu, Japan
b Neil Sherwood Consulting, Nyon, Switzerland

ABSTRACT

Background: Worldwide adoption of electronic cigarettes (e-cigarettes) and heated tobacco products (HTPs) has increased exponentially over the past decade. These products have been proposed as non-combustible alternatives to traditional tobacco products such as cigarettes and may thus reduce the negative health consequences associated with tobacco smoke. However, the overall health impact and safety of using these products remains unclear. This review seeks to provide an updated summary of available evidence on changes to levels of tobacco-related biomarkers to aid the overall assessment of the consequences of using e-cigarettes and HTPs.

Methods: A systematic review was conducted through major databases (Medline/PubMed, Scopus, EMBASE) searching for articles directly comparing biomarker levels in humans using e-cigarettes or HTPs and those using combustible cigarettes. We included peer reviewed articles with comparative or longitudinal design and extracted key information for our purpose (type of population, demographics, biomarkers measurements, and health effects). An initial qualitative analysis was performed followed by a summary of findings.

Results: A total of 44 studies were included from initial citations. The vast majority of the literature reported reductions in levels of biomarkers of tobacco smoke exposure (BOE), especially nicotine, MHBMA, 3-HPMA, S-PMA, 1-OHP and NNAL, when using e-cigarettes and HTPs compared to combustible cigarettes. There was a slight tendency toward a larger reduction in these biomarkers levels with the use of e-cigarettes, although direct comparisons between e-cigarettes and HTPs were lacking. There was also a trend toward positive changes in levels of biomarkers of biological effect (BOBE) with the use of e-cigarettes and HTPs.

Conclusions: A comparison of levels of biomarkers of tobacco-related exposure collected in clinical studies revealed that the use of e-cigarettes and HTPs could lead to a significant reduction in exposure to harmful substances compared to combusted cigarettes. In tandem, the health status of e-cigarette and HTP users, indexed by levels of biomarkers of biological effect showed potential for improvement compared to smoking. However, larger and longer-term population-based studies are needed to further clarify these findings.

1. Introduction

Non-combustible forms of tobacco use, such as electronic cigarettes (e-cigarettes) and heated tobacco products (HTPs) have been emerging and gaining attention in several countries. These products have been proposed as potentially less-risky alternatives to traditional combusted tobacco products such as cigarettes on the basis of reported improvements in levels of biomarkers of tobacco smoke exposure and biological effect, but the long term health impact of these products is still unknown [1]. Because of their worldwide propagation but unclear safety [2],

Abbreviations: BAT, British American Tobacco; BOBE, biomarkers of biological effect; BOE, biomarkers of tobacco smoke exposure; CHTP, Carbon-Heated Tobacco Product; E-cigarettes, electronic cigarettes; EHCSS, Electrically Heated Cigarette Smoking System; EVPs, electronic vapor products; FV, Fontem Ventures; HC, heated cigarette; HTPs, heated tobacco products; JT, Japan Tobacco; mTHS, Menthol Tobacco Heating System; NOS scale, The Newcastle-Ottawa Scale; NSPS, nicotine-salt pod system; NTV, Novel Tobacco vapor products; PMI, Philip Morris International; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RAI, Reynolds American Inc; RCT, randomized controlled trial; RJR, R.J. Reynolds Tobacco Company; RJRVC, R.J. Reynolds Vapor Company; RTP, reduced-toxicant-prototype cigarette; THP, tobacco heating product; THS, Tobacco Heating System; UCS, Uncontrolled smoking conditions; WHO, World Health Organization.

* Corresponding author at: 3-3-41-1202 Orio, Yahatanishi-ku, Kitakyushu, 807-0825, Japan.
E-mail address: y.akiyama55@gmail.com (Y. Akiyama).

1 Two authors contributed equally to the study.

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healthcare authorities have raised various opinions as to the potential health consequences associated with their use and some international institutions have cautioned the need to continuously survey potential adverse events [3]. For example, the World Health Organization (WHO) has aimed to evaluate the health-risks of e-cigarettes [4] and HTPs [5], and proposed strategies to balance their benefits and risks [4,5]. However, to date there has not been any agreement between international healthcare authorities which could expedite a general consensus [1].

Although there are a few epidemiological studies underway examining the long-term impact of e-cigarettes and HTPs on disease endpoints, there are many short-term clinical studies of biomarkers of tobacco smoke exposure (BOE) and biological effect (BOBE) and some systematic literature reviews which have summarized such study results [6,7], including a meta-analysis of BOEs [8] found during the use of HTPs. However, these reviews and meta-analyses have considered the results of either e-cigarettes or HTPs separately, and did not consistently address the results of clinical studies on biomarkers of biological effect (BOBE) that many consider to lie on the pathway to smoking-related diseases.

In the light of this heterogenous evidence, and to examine suggestions that e-cigarettes and HTPs can serve as less-risky alternatives to conventional tobacco products, we aimed to survey and summarize differences in both BOE and BOBE during use of either e-cigarettes or HTPs compared to the use of conventional tobacco products such as cigarettes.

2. Methods

This is a systematic review conducted in accordance with recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [9].

2.1. Search strategy and selection criteria

We conducted a systematic review of the literature within three main electronic databases (Medline/PubMed, Scopus, EMBASE) to identify all articles comparing biomarkers between human beings exposed to e-cigarettes / HTPs and smoking. Literature search was conducted using the electronic search strategy: ["e-cigarette" OR "electronic cigarette" OR "e-vapor"] AND ("biomarker" OR "trial") OR ["heated tobacco" OR "heat not burn" OR "heat-not-burn" OR "tobacco heating" OR "IQOS" OR "Ploom" OR "glo" OR "novel tobacco ") AND ("biomarker" OR "trial") from inception until April 15 of 2020 and was restricted to peer reviewed articles published in English. The search strategy was translated in accordance to the other database Boolean operators. We also searched cross-references to complement the evidence given in this review. The main types of studies included were randomized trials, case-control studies, and cohort studies. Design of the studies could be either comparative (e-cigarettes/HTPs users, smokers, non-smokers/past smokers) or longitudinal with a switch from smoking to e-cigarettes or HTPs. Publications were excluded if they were conducted in vitro or in vivo, written in languages other than English or not peer reviewed.

2.2. Data extraction

The title and abstract were screened by two reviewers independently to confirm the inclusion criteria. The full text of the selected articles was retrieved, and each reference list was screened to identify additional publications on this topic. Any discrepancies in the selected studies were solved by a third reviewer. Selected articles were stratified into two groups: (1) studies comparing biomarkers of exposure between e-cigarettes/HTPs and conventional smoking, (2) studies comparing...
### Studies included in the review.

| Authors, year of publication [Reference] | Affiliation | Study location | Study design | Product Name [Reference product] | Intervention period |
|------------------------------------------|-------------|----------------|--------------|-----------------------------------|--------------------|
| **HTPs RCT studies on biomarker of exposure (Table 2)** |             |                |              |                                   |                    |
| Ludicke et al., 2016 [12]                | PMI         | Poland         | RCT          | GHTP (Cigarette)                  | 5 days             |
| Haziza et al., 2016 [13]                 | PMI         | Japan          | RCT          | THS 2.2 (Cigarette)               | 5 days             |
| Haziza et al., 2017 [14]                 | PMI         | Poland         | RCT          | THS 2.2 (Cigarette)               | 5 days             |
| Ludicke et al., 2018b [16]               | PMI         | Japan          | RCT          | mTHS (menthol Cigarette)          | 5 days             |
| Walele et al., 2018 [38]                 | Fontem Ventures U.S.A. RCT | Puritane     |              |                                   |                    |
| Jay et al., 2020 [32]                   | JUUL Labs   | Japan          | RCT          | JUUL NSPS (Cigarette)             | 5 days             |
| Pulvers et al., 2020 [35]               | JT          | Japan          | RCT          | EHCSS-K6m (menthol Cigarette)     | 6 days             |
| Haziza et al., 2020a [17]                | PMI         | Japan          | RCT          | mTHS (menthol Cigarette)          | 5 days             |
| Yuki et al., 2018 [18]                  | JT          | Japan          | RCT          | NTV (Cigarette)                   | 5 days             |
| Tricker et al., 2012c [19]              | PMI         | Japan          | RCT          | EHCSS-K6 (Cigarette)              | 6 days             |
| Gale et al., 2019 [20]                  | BAT         | Japan          | RCT          | iQOS/THP1.0 (Cigarette)           | 6–7 days           |
| Roethig et al., 2007 [65]               | PM USA      | –              | RCT          | EHCSS -UCS (Cigarette)            | 8 days             |
| Frost-Pineda et al., 2008a [66]         | PM USA      | –              | RCT          | EHCSS (Cigarette)                 | 8 days             |
| Roethig et al., 2005 [21]               | PM USA      | U.S.A.         | RCT          | EHCSS1 (Cigarette)                | 8 days             |
| Tricker et al., 2012b [22]              | PMI         | Japan          | RCT          | EHCSS-K3 (Cigarette)              | 8 days             |
| Martin Leroy et al., 2012 [23]          | PMI         | Poland         | RCT          | EHCSS-K6 (Cigarette)              | 8 days             |
| Tricker et al., 2012d [24]              | PMI         | UK             | RCT          | EHCSS-K3 (Cigarette)              | 8 days             |
| Tricker et al., 2012a [25]              | PMI         | UK             | RCT          | EHCSS-K6 (Cigarette)              | 8 days             |
| Sakaguchi et al., 2014 [26]             | JT          | Japan          | RCT          | HC (Cigarette)                    | 28 days            |
| Frost-Pineda et al., 2008b [67]         | PM USA      | U.S.A.         | RCT          | EHCSS (Cigarette)                 | 12 weeks           |
| Ludicke et al., 2019 [27]               | PMI         | U.S.A.         | RCT          | THS 2.2 (Cigarette)               | 3 months           |
| Shepard et al., 2015 [28]               | BAT         | Germany        | RCT          | RTP (Cigarette)                   | 6 months           |
| Ogden et al., 2015a [29]                | RAI, RJR    | U.S.A.         | RCT          | Eclipse (Cigarette)               | 24 weeks           |
| Roethig et al., 2008 [68]               | PM USA      | –              | RCT          | EHCSS (Cigarette)                 | postbaseline (~12 months) |
| E-cigarettes RCT studies on biomarker of exposure (Table 3) |             |                |              |                                   |                    |
| O’Connell et al., 2016 [30]             | Fontem Ventures | U.S.A.     | RCT          | Blu (Cigarette)                   | 5 days             |
| Round et al., 2019 [31]                 | RJR VC      | U.S.A.         | RCT          | Vuse Solo (Cigarette)             | 5 days             |
| Jay et al., 2020 [32]                   | JUUL Labs   | U.S.A.         | RCT          | menthol Vuse Solo (menthol Cigarette) | 5 days |
| Goniewicz et al., 2017 [33]             | Department of Health Behavior, Roswell Park Cancer Institute | Poland        | RCT          | M201 Mild (Cigarette)             | 2 weeks            |
| McRobbie et al., 2015 [34]              | Tobacco Dependence Research Unit & UK Centre for Tobacco and Alcohol Studies, Wolfson Institute | UK          | RCT          | Green Smoke EC (Cigarette)        | 4 weeks            |
| Pulvers et al., 2018 [35]               | Department of Psychology, California State University San Marcos | U.S.A.         | RCT          | e-Go C (Cigarette)                | 4 weeks            |
| Hatsukami et al., 2019 [36]             | Department of Psychiatry, University of Minnesota | U.S.A.         | RCT          | Vuse Solo Blu cigarettes Fin(Cigarette) | 8 weeks |
| Cravo et al., 2016 [37]                 | Fontem Ventures | UK          | RCT          | EVP (Cigarette)                   | 12 weeks           |
| Walele et al., 2018 [38]                | Fontem Ventures | U.S.A.     | RCT          | Puritane™ (Cigarette)             | 24 months          |
| E-cigarettes cross sectional studies on biomarker of exposure (Table 4) |             |                |              |                                   |                    |
| Shahab et al., 2017 [39]                | Department of Epidemiology and Public Health, University College London | UK          | Cross Sectional | E-cigarettes (Cigarette)          | –                  |
| Goniewicz et al., 2018 [40]             | Department of Health Behavior, Roswell Park Comprehensive Cancer Center | U.S.A. | Cross Sectional | E-cigarettes (Cigarette)          | –                  |
| Oliveri et al., 2020 [41]               | Atria        | U.S.A.         | Cross Sectional | EVP (Cigarette)                  | –                  |
| Ye et al., 2020 [42]                    | Eastman Institute for Oral Health, University of Rochester Medical Center | U.S.A. | Cross Sectional | Electronic cigarettes (Cigarette) | –                  |
| Lockiewicz et al., 2019 [43]            | American Heart Association | U.S.A. | Cross Sectional | Electronic cigarettes (Cigarette) | –                  |
| Bustamante et al., 2018 [44]            | Division of Environmental Health Sciences, University of Minnesota | U.S.A. | Cross Sectional | Electronic cigarettes            | –                  |
| Ghosh et al., 2019 [45]                 | Marisco Lung Institute | U.S.A.  | Cross Sectional | E-cigarettes (Cigarette)          | –                  |
| HTPs and E-cigarettes RCT studies on biomarker of effect (Table 5) |             |                |              |                                   |                    |
| Martin Leroy et al., 2012 [23]          | PMI         | Poland         | RCT          | EHCSS-K6 (Cigarette)              | 35 days            |
| Ludicke et al., 2018a [46]              | PMI         | Japan          | RCT          | mTHS (menthol Cigarette)          | 90 days            |
| Haziza et al., 2020b [47]               | PMI         | U.S.A.         | RCT          | nTHS 2.2 (methol Cigarette)       | 3 months           |

(continued on next page)
biomarkers of biological effect between e-cigarettes/HTPs and conventional smoking. We extracted clinical information such as the study design, demographic characteristics, and type of biomarker. Lastly, the sample size and the levels of biomarkers were obtained for each study.

### 2.3. Study assessment

The methodological quality was assessed using the Cochrane bias components (used for randomized trials) also known as six domains (selection, performance, detection, attrition, reporting, and other) each one sum 2 point if low risk, 1 point if unclear risk or 0 if high risk [10]. The Newcastle-Ottawa Scale (NOS) was used for observational studies [11], which is a scale that ranges from 0 to 8 and considers the following aspects: representativeness of the exposed cases/cohort, selection of non-exposed group, outcome ascertainment, outcome not present at baseline, comparability between groups, outcome assessment, follow-up long enough, non-response rate [11]. Those studies with score ≥ 3 were considered of moderate quality.

### 3. Results

#### 3.1. Literature search results

Initially the literature search yielded 2091 citations, of which 1319 studies remained after 772 duplicates were removed. An additional 1185 articles were removed based on a title or abstract that was not relevant according to the inclusion criteria. Subsequent full-text screening resulted in exclusion of another 70 articles, leaving us with a total of 64 articles. Cross-reference checking did not reveal any additional articles missed by the search strategy. Of the 44 publications that met the inclusion and exclusion criteria for data extraction and final analyses (Fig. 1) [12–51,65–68], 25 articles for HTPs [12–29,46–48, 65–68], and 19 for e-cigarettes were identified [30–45,49,13–51]. With some overlap, 38 articles for biomarkers of exposure and 14 for biomarkers of biological effect were identified. 12 publications were considered as independent studies, and 32 manufacturer-funded studies. Table 1 summarizes the characteristics of the studies included in this systematic review.

#### 3.2. Study assessment

Overall the quality of the studies was moderate/good. All trials included in this systematic review had a moderate/high methodological quality according to the Cochrane tool which considered five domains for assessing the risk of bias. The cross-sectional studies included in this review had mostly moderate methodological quality according to the NOS scale (median 5, interquartile range 4–6) which considered eight domains explained previously.

#### 3.3. Biomarkers of exposure (BOE)

Supplementary Table 1 shows the list of biomarkers of exposure and corresponding constituents. For HTPs, there were 30 trials comparing BOE profiles with combustible cigarettes, with a median intervention period of 8 days (range from 5 days to 12 months). The most common studied BOEs were COHb, MHBMA, 4-ABP, 3-HPMA, S-PMA, o-Toluidine, NEQ and 1–OHP. The levels of all of these biomarkers were significantly reduced after switching from a conventional cigarette to HTPs, and on average the reductions in the levels of biomarkers exceeded half of the baseline values. All trials showed reductions in most of the measured biomarkers. In some studies nicotine and cotinine biomarker concentrations increased (when the data was available) whereas in others they decreased. It is possible that differences between products in their nicotine content and release, and/or changes to user behaviour on switching to HTPs may account for these divergent results. Table 2a and 2b provides more details and BOE comparisons of the studies on HTPs.

For e-cigarettes, a total of 10 trials were included comparing BOE profiles between e-cigarettes and combustible cigarettes. The median follow-up period was 2 weeks (range from 5 days to 12 weeks). Carbon monoxide, MHBMA, CEMA, 3-HPMA, S-PMA, HMPMA, NEQ, NNAL and NNN were the most frequently studied BOEs. The levels of all these biomarkers were consistently reduced from their baseline value. In some studies nicotine and cotinine biomarker concentrations increased (when the data was available) whereas in others they decreased. It is possible that differences between products in their nicotine content and release, and/or changes to user behaviour on switching to HTPs may account for these divergent results. Table 3 shows more details and biomarker comparisons of the studies on e-cigarettes. 7 cross sectional studies also demonstrated a consistent and significant decrease in some BOEs (CEMA, GAMA, HEMA, 2MHA, NNAL) as shown in Table 4. In one study [43] the 1,3-butanediol metabolite MHBMA2 showed an increase of 1200 %, while all other related metabolites (DH MBA, MHBMA1, and MHBMA3) decreased in the same study. It was unclear why only MHBMA2 increased so significantly. The authors of the original study did not discuss this result in detail and it appears no data were collected which could help validate this finding, such as, 1,3-butanediol levels in the mainstream e-cigarette aerosol.

#### 3.4. Biomarkers of biological effect (BOBE)

Supplementary Table 2 shows the list of biomarkers of effect and corresponding effects. Regarding BOBE, the results show that levels found during the use of both e-cigarettes and HTPs were generally

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**Table 1** (continued)

| Authors, year of publication [Reference] | Affiliation | Study location | Study design | Product Name (Reference product) | Intervention period |
|----------------------------------------|-------------|----------------|--------------|-----------------------------------|-------------------|
| Ludicke et al., 2019 [27]             | PMI         | U.S.A.          | RCT          | THS 2.2 (Cigarette)              | 3 months          |
| Shaper et al., 2015 [28]              | BAT         | Germany         | RCT          | THS 2.2 (Cigarette)              | 6 months          |
| Ogden et al., 2015b [48]              | RAI, RJR     | U.S.A.          | RCT          | RTP (Cigarette)                 | 6 months          |
| Roethig et al., 2008 [68]             | PM USA       | –              | RCT          | EHCSS (Cigarette)               | postbaseline (<12 months) |
| D’Raúz et al., 2017 [49]              | Fontem Ventures | U.S.A.      | RCT          | blu (Cigarette)                | 5 days            |
| Cravo et al., 2016 [57]               | Fontem Ventures | UK          | RCT          | EVP (Cigarette)                | 12 weeks          |

**Table 2a**

| Study | Product Name (Reference product) | Intervention period |
|-------|----------------------------------|-------------------|
| PMI U.S.A. RCT THS 2.2 (Cigarette) | 3 months          |
| PMI U.S.A. RCT THS 2.2 (Cigarette) | 6 months          |
| Germany RCT RTP (Cigarette)       | 6 months          |
| U.S.A. RCT Eclipse (Cigarette)    | 24 weeks          |
| PM USA – RCT EHCSS (Cigarette)    | postbaseline (<12 months) |
| U.S.A. RCT blu (Cigarette)       | 5 days            |
| UK RCT EVP (Cigarette)           | 12 weeks          |

**Table 2b**

| Study | Product Name (Reference product) | Intervention period |
|-------|----------------------------------|-------------------|
| Comprehensive Cancer Center, The Ohio State University and James Cancer Hospital | Cross Sectional E-cigarettes (Cigarette) |
| Eastman Institute for Oral Health, University of Rochester Medical Center | Cross Sectional Electronic cigarettes (Cigarette) |
| Arial | Cross Sectional EVP (Cigarette) |
| Marsico Lung Institute | Cross Sectional E-cigarettes (Cigarette) |
| Ohio State Wexner Medical Center | Cross Sectional E-cigarettes (Cigarette) |

**Table 3**

| Study | Product Name (Reference product) | Intervention period |
|-------|----------------------------------|-------------------|
| Comprehensive Cancer Center, The Ohio State University and James Cancer Hospital | Cross Sectional E-cigarettes (Cigarette) |
| Eastman Institute for Oral Health, University of Rochester Medical Center | Cross Sectional Electronic cigarettes (Cigarette) |
| Arial | Cross Sectional EVP (Cigarette) |
| Marsico Lung Institute | Cross Sectional E-cigarettes (Cigarette) |
| Ohio State Wexner Medical Center | Cross Sectional E-cigarettes (Cigarette) |

**Table 4**

| Study | Product Name (Reference product) | Intervention period |
|-------|----------------------------------|-------------------|
| Comprehensive Cancer Center, The Ohio State University and James Cancer Hospital | Cross Sectional E-cigarettes (Cigarette) |
| Eastman Institute for Oral Health, University of Rochester Medical Center | Cross Sectional Electronic cigarettes (Cigarette) |
| Arial | Cross Sectional EVP (Cigarette) |
| Marsico Lung Institute | Cross Sectional E-cigarettes (Cigarette) |
| Ohio State Wexner Medical Center | Cross Sectional E-cigarettes (Cigarette) |

**Table 5**

| Study | Product Name (Reference product) | Intervention period |
|-------|----------------------------------|-------------------|
| Comprehensive Cancer Center, The Ohio State University and James Cancer Hospital | Cross Sectional E-cigarettes (Cigarette) |
| Eastman Institute for Oral Health, University of Rochester Medical Center | Cross Sectional Electronic cigarettes (Cigarette) |
| Arial | Cross Sectional EVP (Cigarette) |
| Marsico Lung Institute | Cross Sectional E-cigarettes (Cigarette) |
| Ohio State Wexner Medical Center | Cross Sectional E-cigarettes (Cigarette) |

**Table 6**

| Study | Product Name (Reference product) | Intervention period |
|-------|----------------------------------|-------------------|
| Comprehensive Cancer Center, The Ohio State University and James Cancer Hospital | Cross Sectional E-cigarettes (Cigarette) |
| Eastman Institute for Oral Health, University of Rochester Medical Center | Cross Sectional Electronic cigarettes (Cigarette) |
| Arial | Cross Sectional EVP (Cigarette) |
| Marsico Lung Institute | Cross Sectional E-cigarettes (Cigarette) |
| Ohio State Wexner Medical Center | Cross Sectional E-cigarettes (Cigarette) |
### Table 2a

| Studies | RCT studies on biomarker of exposure, % change from baseline |
|---------|-------------------------------------------------------------|
| Affiliation | PMI PMI PMI PMI PMI PMI JT PMI BAT BAT BAT BAT PM USA PM USA PMI PMI |
| Study location | PL JP PL PL JP US JP JP JP JP JP JP US JP JP JT BAT BAT BAT BAT |
| Product Name | GHTP THS 2.2 THS 2.2 THS 2.1 mTHS mTHS NTV EHCSS- glo/THP mglo/THP iQOS/THS EHCSS- EHCSS EHCSS1 EHCSS2 |
| Reference product | (Cig) (Cig) (Cig) (Cig) (mCig) (mCig) (Cig) (Cig) (mCig) (mCig) 1.0 (Cig) 1.0 (mCig) (Cig) (Cig) (Cig) (Cig) |

| End of the study | p | d-d | d | d | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
|------------------|---|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CO               | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| COHs             | −59.7 | −51.13 | −76.20 | −75.79 | −51.46 | −64.41 | −50.86 | −50.86 | −85.08 | −85.64 | −50.86 | −50.86 | −85.08 | −85.64 | −50.86 | −50.86 |
| MIBMA            | −86.7 | −66.41 | −84.98 | −86.71 | −87.50 | −92.02 | −89.68 | −89.68 | −91.32 | −91.32 | −89.47 | −89.47 | −84.30 | −84.30 | −63.8 | −63.8 |
| DHBMA            | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| p                | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| HBMA             | −74.8 | −74.08 | −82.12 | −75.11 | −78.88 | −83.64 | −86.56 | −86.56 | −80.57 | −80.57 | −81.89 | −81.89 | −78.26 | −78.26 | −59.8 | −59.8 |
| THSS             | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| CEMA             | −79.42 | −86.10 | −85.62 | −83.49 | −84.12 | −87.21 | nd | nd | −89.23 | −87.80 | −87.17 | nd | nd | nd | nd | nd |
| mTHSS            | −70.6 | −47.33 | −49.68 | −66.89 | −54.35 | −60.63 | −53.00 | −27.9 | −52.95 | −48.74 | −37.42 | −48.01 | nd | nd | nd | nd |
| mTHSS2           | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| THS-1            | −82.2 | −72.24 | −92.03 | −90.59 | −88.82 | −91.15 | −89.51 | −89.13 | −89.13 | −89.48 | −89.78 | −85 | nd | nd | nd | nd |
| THS-2            | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| THS-3            | −69.75 | −89.22 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| THS-4            | 67.98 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |

**References:** [12-14, 19, 21, 22, 24, 25, 65-68].
### Table 2b

**HTTPs RCT studies on biomarker of exposure, % change from baseline**

| Affiliation | [22] | [22] | [23] | [24] | [24] | [24] | [25] | [26] | [67] | [17] | [16] | [27] | [27] | [28] | [29] | [68] |
|-------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Study location | PMI | PMI | PMI | PMI | PMI | PMI | JT | PM USA | PMI | PMI | PMI | PMI | BAT | RAI | RJR | PM USA |
| Product Name | EHCSS-K3 | EHCSS-K6 | EHCSS-K6 | EHCSS-K6 | EHCSS-K6 | EHCSS-K6 | HC | EHCSS | mTHS | mTHS | TTHS 2.2 | TTHS 2.2 | RTP | Eclipse | EHCSS (Cig) |
| (Reference product) | (Cig) | (Cig) | (Cig) | (Cig) | (Cig) | (Cig) | (mCig) | (mCig) | (Cig) | (Cig) | (Cig) | (Cig) | (Cig) | (Cig) | (Cig) |
| End of the study | 8 d | 8 d | 8 d | 8 d | 8 d | 8 d | 28 d | 12 w | 90 d | 90 d | 3 m | 6 m | 24 w | postbaseline |
| p | <.001 | <.001 | <.001 | <.05 | <.05 | <.05 | <.05 | nd | nd | nd | nd | <.001 | nd | nd | nd |
| COHb | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| MHBMA | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 4-ABP | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 3-HMPMA | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| o-Toluidine | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 1-NAP | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2-cyanoethylvaline Hb | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2-NA | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| NEQ | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Cotinine | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Rf | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 1-NA | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2-NAP | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Total OH Naphthalene | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 1-OP | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| NNK | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |

*Gig, cigarette; d, days; DE, Germany; JP, Japan; KR, Republic of Korea; mCig, menthol cigarette; m, months; nd, no data; ns, not significant; PL, Poland; UK, United Kingdom; US, United States of America; w, weeks; *Calculated in tow ways. 1) Calculated by averaging the rate of change from baseline in individual subjects. [12-14,19,21,22,24,25,65-68] 2) Calculate by using the mean (arithmetic mean, geometric mean, LS mean) or median of each marker at baseline and last day. [15-18,20,23,26,27]
moved in a direction believed to be consistent with improved health outcomes (Tables 5, 6). 10 trials and 5 cross sectional studies assessed the effects of BOBE changes, with a follow up period ranging from 5 days to 12 months. Those studies measured a total of 90 BOBEs in blood, urine or saliva, including markers related to clinical laboratory test (13 markers), inflammation/oxidative damage (52 markers), lipids (6 markers), hypercoagulable state (7 markers), growth factors (11 markers). Additional biomarkers of tobacco smoke exposure (BOE) and biological effect markers, hypercoagulable state (7 markers), growth factors (11 markers), and tissue injury and repair (1 marker).

The most consistent finding across the studies was the reduction in the levels of thromboxane (11-DTX-B2) by 10–30 % and white blood cells between 0–13 % from baseline. There were also some benefits in terms of lipid profile, showing an increase of HDL and reduction of LDL. Other BOBEs which showed reduction in multiple studies were FEV1% predicted, Systolic blood pressure, Diastolic blood pressure, 812-iso-IPF2α, VI, 8-epi-PGF2α, sICAM1, CRP, Neutrophil count, OxLDL, Triglycerides, Fibrinogen and Hgb (Table 5).

Additionally, 5 cross sectional studies favoured the use of e-cigarettes over combustible cigarettes, demonstrating better profiles for oxidative damage and growth factors (Table 6), which included a reduction in levels of 8-epi-PGF2α, sICAM1, 11-DTX-B2, macrophages and IL18. There was only one study that measured and recorded significant differences regarding growth factors [42]. (Table 6).

4. Discussion

This systematic review identified clinical studies which had examined biomarkers of tobacco smoke exposure (BOE) and biological effect (BOBE) during the use of e-cigarettes and HTPs, taken from major literature databases. The results provide elemental insights for a critical appraisal of e-cigarettes and HTPs as alternatives to combustible tobacco products such as cigarettes. Taken together, all findings suggest that BOE levels measured in users of e-cigarettes and HTPs show a significant
reduction compared to a cigarette condition (or cigarette baseline).

There is also some evidence to suggest that e-cigarette users are exposed to fewer harmful substances overall, and in lower concentrations, than users of HTPs.

We studied the majority of biomarkers of exposure associated with tobacco. There are numerous substances of concern and related biomarkers based on the list of priority toxicants proposed by the WHO Study Group on Tobacco Product Regulation. Most of them have been widely studied due to their potential link to smoking-related health risks [52–54]. Our biomarker findings imply that the majority of toxicants are emitted in lower amounts (if at all) from e-cigarettes and HTPs compared to combusted tobacco products such as cigarettes. This is consistent with the results of research on mutagenicity, which has been used as an indicator of the genetic mutagenic potential of substances present in human urine [55].

Relevant biomarker levels in users of e-cigarettes and HTPs were indicative of reduced exposure to butadiene, acrolein, benzene, toluene, naphthylamine and methyl nitrosamines. Most of these chemicals are considered carcinogenic and hazardous for human health. For example, according to the United States Environmental Protection Agency, butadiene is a potent carcinogen that is also derived from motor vehicle exhaust and is known to increase the risk of cardiovascular diseases, leukemia and lung irritation [56]. Similarly, other authorities have also suggested that toxicants like acrolein or benzene may cause respiratory tract irritation as well as gastrointestinal mucosa hyperplasia.

### Table 4

E-cigarettes cross sectional studies on biomarker of exposure, % difference between cigarettes.

| References | [39] | [40] | [41] | [42] | [43] | [44] | [45] |
|------------|------|------|------|------|------|------|------|
| Affiliation | independent | independent | Altria | independent | independent | independent | independent |
| Study location | UK | US | US | US | US | US | US |
| Product Name (Reference product) | E-cig (Cig) | E-cig (Cig) | EVP (Cig) | E-cig (Cig) | E-cig (Cig) | E-cig (Cig) | E-cig (Cig) |
| p | <.001 | <.05 | <.001 | nd | nd | nd | nd |
| COHb | nd | nd | –46.34 | nd | nd | nd | nd |
| BPM | 15.62 | nd | –70.32 | nd | nd | nd | nd |
| DHBM | –27.93 | nd | nd | nd | nd | nd | nd |
| DHBMA | –22.89 | nd | nd | nd | –5.94 | nd | nd |
| MBH3 | –84.55 | nd | nd | nd | nd | nd | nd |
| MBHMA1 | nd | nd | –100.00 | nd | nd | nd | nd |
| MBHMA2 | nd | nd | 1200.00 | nd | nd | nd | nd |
| MBHMA3 | –85.10 | nd | nd | nd | –52.44 | nd | nd |
| TTCA | ns | ns | nd | nd | –93.34 | nd | nd |
| Acetate | nd | nd | nd | nd | 46.88 | nd | nd |
| CEMA | –54.42 | –60.22 | nd | nd | –83.30 | nd | nd |
| 3-HPMA | –64.10 | nd | –45.95 | nd | –38.95 | nd | nd |
| HPMA | nd | –72.47 | nd | nd | nd | nd | nd |
| AAMA | –55.33 | –58.90 | nd | nd | 61.37 | nd | nd |
| GAMA | –45.94 | –42.73 | nd | nd | –85.68 | nd | nd |
| AMCA | nd | –68.15 | nd | nd | nd | nd | nd |
| CVHA | nd | –88.84 | nd | nd | nd | nd | nd |
| CYMA | –97.15 | –96.80 | nd | nd | 31.81 | nd | nd |
| HEMA | –48.14 | –60.78 | nd | nd | –100.00 | nd | nd |
| TMA | ns | nd | nd | nd | 69.87 | nd | nd |
| HPMM | nd | –81.23 | nd | nd | nd | nd | nd |
| HPMAA | –70.66 | nd | nd | nd | –22.95 | nd | nd |
| ATCA | ns | nd | nd | nd | 28.11 | nd | nd |
| AMCC | –62.51 | nd | nd | nd | –14.92 | nd | nd |
| PGHA | ns | –40.47 | nd | nd | 49.81 | nd | nd |
| Formate | nd | nd | 96.62 | nd | nd | nd | nd |
| IPM3 | nd | –88.81 | nd | nd | nd | nd | nd |
| HPM2 | nd | –51.54 | nd | nd | nd | nd | nd |
| 2HPMA | –28.71 | nd | nd | nd | –58.52 | nd | nd |
| PHGMA | ns | nd | nd | nd | –50.00 | nd | nd |
| MADA | –46.55 | –50.41 | nd | nd | 4.95 | nd | nd |
| S-BMA | nd | ns | nd | nd | –77.27 | nd | nd |
| 1,2DCVMA | nd | nd | nd | nd | –76.11 | nd | nd |
| 2,2DCVMA | nd | nd | nd | nd | –100.00 | nd | nd |
| 2MHA | –74.94 | –71.88 | nd | nd | –64.98 | nd | nd |
| 3MHA + 4MHA | –80.71 | –72.71 | nd | nd | 59.82 | nd | nd |
| NEQ | ns | –92.83 | ns | nd | nd | nd | nd |
| NICT | ns | –60.63 | nd | nd | –96.40 | 57.53 | –44.67 |
| Cotinine | ns | –93.21 | nd | nd | 111.94 | 7.69 | –43.45 |
| HCCTT | ns | –92.85 | nd | nd | –6.98 | nd | nd |
| CONT | ns | –60.69 | nd | nd | nd | nd | nd |
| NOXT | ns | –56.09 | nd | nd | nd | nd | nd |
| NCC | ns | –64.72 | nd | nd | nd | nd | nd |
| NNCT | ns | –68.72 | nd | nd | –29.51 | nd | nd |
| 1-NAP | nd | –86.04 | nd | nd | nd | nd | nd |
| 2-NAP | nd | –61.99 | nd | nd | nd | nd | nd |
| 1-Hydroxypropane | nd | –46.86 | nd | nd | nd | nd | nd |
| NNAL | –97.24 | –97.59 | –86.26 | nd | nd | –98.01 | nd |
| NAB | –82.65 | –90.92 | nd | nd | nd | nd | nd |
| NAT | –94.54 | –95.93 | nd | nd | nd | nd | nd |
| NNN | nd | –70.58 | nd | nd | –99.66 | nd | nd |

Cig, cigarette; d, days; DE, Germany; JP, Japan; KR, Republic of Korea; mCig, menthol cigarette; m, months; nd, no data; ns, not significant; PL, Poland; UK, United Kingdom; US, United States of America; w, weeks;

* Calculate by using the mean (arithmetic mean, geometric mean, LS mean) of each marker on e-cigarette group and cigarette group.
Table 5
HTPs and E-cigarettes RCT studies on biomarker of effect, % change from baselinea,b.

| References | [23] | [46] | [47] | [27] | [27] | [28] | [48] | [68] | [49] | [37] |
|------------|------|------|------|------|------|------|------|------|------|------|
| Affiliation | PMI  | PMI  | PMI  | PMI  | BAT  | RAI, RJR | PM USA | FV  | FV  | FV  |
| Study location | PL   | JP   | US   | US   | US   | DE   | US   | –   | US  | UK  |
| Product type | HTPs | HTPs | HTPs | HTPs | HTPs | HTPs | HTPs | –   | –   | –   |
| Product Name (Reference product) | EHCS-K6 | mTHS | mTHS 2.2 | THS 2.2 | THS 2.2 | RTP | Eclipse | EHCSS (Cig) | blu | EVP |
| End of Study | 35 d | 90 d | 3 m  | 3 m  | 6 m  | 6 m  | 24 w | postbaseline | 5 d  | 12 w |
| p | <.001 | nd   | nd   | nd   | nd   | <.001 | <.05 | nd   | nd   |
| Clinical laboratory test | | | | | | | | | |
| FEV1 speed | nd | 1.55 | nd | –0.62 | –1.46 | nd | nd | nd | 6.0 | nd |
| FVC | nd | nd | nd | nd | nd | nd | nd | nd | 1.9 | nd |
| CEP | nd | nd | nd | nd | nd | nd | nd | nd | 55 | nd |
| HgBA1C | nd | 11.35 | 9.27 | nd | nd | –1 | nd | nd | |
| Homocysteine | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| SCE | nd | nd | nd | nd | nd | –3 | nd | nd | |
| RBC count | –2.22 | nd | nd | nd | nd | nd | 0.00 | nd | nd ||
| Glucose | nd | 5.77 | nd | 0.96 | nd | nd | nd | nd | nd |
| Body weight | nd | 0.51 | nd | nd | nd | nd | nd | nd | nd |
| Waist circumference | nd | 5.77 | nd | nd | nd | nd | nd | nd | nd |
| Systolic blood pressure | nd | –7.00 | nd | nd | nd | nd | nd | nd | nd |
| Diastolic blood pressure | nd | –5.44 | nd | nd | nd | nd | nd | nd | –6.0 |
| Hemoglobin | nd | –6.26 | nd | nd | nd | nd | nd | nd | –5.7 |
| Heat rate | nd | nd | nd | nd | nd | nd | nd | nd | –7.2 |
| Lipids | | | | | | | | | |
| Total cholesterol | 1.47 | nd | nd | nd | nd | nd | nd | nd | nd |
| Triglycerides | | | | | | | | | |
| MPO | –2.01 | nd | nd | nd | nd | nd | nd | nd | nd |
| Catalase activity to Hb ratio | nd | nd | nd | nd | nd | –79.8 | nd | nd | nd |
| Malondialdehyde to Hb ratio | nd | nd | nd | nd | nd | 81.0 | nd | nd | nd |
| Ascorbic acid | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Dehydroascorbic acid | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Total antioxidant capacity | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Neutrophil elastase | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| LTB4 | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Theophylline | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Neutrophil count | –5.12 | nd | nd | nd | nd | nd | nd | nd | nd |
| Lymphocytes | –1.66 | nd | nd | nd | nd | nd | nd | nd | nd |
| Monocyte count | 0.00 | nd | nd | nd | nd | nd | nd | nd | nd |
| Total IgM | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Lipids | | | | | | | | | |
| HDL | 10.52 | 5.97 | nd | 0.73 | 0.73 | 8.0 | 0 | 10.81 | nd | 0.56 |
| LDL | –4.91 | –6.51 | nd | nd | nd | 2.1 | –1 | –0.88 | nd | –1.69 |
| HDL/LDL | nd | nd | nd | nd | nd | nd | nd | 2 | nd | nd |
| OxLDL | 60.76 | nd | nd | nd | nd | –3.7 | 2 | nd | nd | nd |
| Triglycerides | nd | –0.71 | nd | nd | nd | –4.2 | 15 | 3.50 | nd | nd |
| Total cholesterol | 1.47 | –3.24 | nd | nd | nd | 2.7 | nd | nd | nd | nd |
| Hypercoaguable state | | | | | | | | | |
| Fibrinogen | 6.06 | –1.17 | –5.94 | nd | nd | –1.3 | –1 | –3.77 | nd | nd |
| Platelets | 0.80 | nd | nd | nd | nd | nd | nd | nd | nd |
| HCT | –2.81 | nd | nd | nd | nd | nd | nd | –1.66 | nd | nd |
| Hgb | –2.09 | nd | nd | nd | nd | nd | nd | –1.38 | nd | –1.27 |
| vWF | –11.11 | nd | nd | nd | nd | nd | nd | –4.72 | nd | nd |
| ADP-induced platelet aggregation: slope | 0.86 | nd | nd | nd | nd | nd | nd | nd | nd |
| ADP-induced platelet aggregation: amplitude (%) | 1.28 | nd | nd | nd | nd | nd | nd | nd | nd |

Cig, cigarette; d, days; DE, Germany; JP, Japan; KR, Republic of Korea; mCig, menthol cigarette; m, months; nd, no data; ns, not significant; PL, Poland; UK, United Kingdom; US, United States of America; w, weeks;
Globally it is understood that smoke-related diseases are consequences of pathophysiological processes that involve oxidative stress and chronic inflammation [69]. It is therefore hypothesized that a favorable change in BOBEs, comprising variables related to lipid metabolism, endothelial function, inflammation, oxidative stress, platelet activation, and pulmonary function, could potentially contribute to improved health outcomes. In particular, some of the BOBE which showed significant level changes in this review (sICAM-1, WBC, 11-DHTXB2 and 8-epi-PGF2α) have been reported as associated with smoking-related diseases such as CVD [57–63]. However, this is still a fertile area of research with some topics that need to be clarified such as the real health benefits that may results from the conversion to 

| Table 6 | E-cigarettes cross sectional studies on biomarker of effect, % difference between cigarettes\(a,b\). |
|----------|--------------------------------------------------|
| References | [50] | [42] | [41] | [45] | [51] |
| Affiliation | independent | independent | Altria | independent | independent |
| Study location | US | US | US | US | US |
| Study design | Cross Sectional | Cross Sectional | Cross Sectional | Cross Sectional | Cross Sectional |
| Product type | E-cig | E-cig | E-cig | E-cig | E-cig |
| Product Name (Reference product) | E-cig (Cig) | E-cig (Cig) | EVP (Cig) | E-cig (Cig) | E-cig (Cig) |
| p | <.05 | nd | <.05 | nd | nd |
| Clinical laboratory test | | | | | |
| FEV1%pred | nd | nd | nd | –6.67 | nd |
| FVC | nd | nd | nd | –16.91 | nd |
| Inflammation/Oxidative damage | | | | | |
| 8-epi-PGF2α | nd | nd | –22.85 | nd | nd |
| sICAM1 | nd | nd | –15.72 | nd | nd |
| WBC | nd | nd | –8.69 | nd | nd |
| 11-DTX-B2 | nd | nd | –29.09 | nd | nd |
| Neutrophil count | –70.00 | nd | nd | –70.00 | nd |
| Lymphocytes | 30.00 | nd | nd | 30.00 | nd |
| Eosinophils | nd | nd | nd | 42.50 | nd |
| Macrophages | –35.52 | nd | nd | –1.60 | –35.52 |
| Polymorphonuclear cells | nd | nd | nd | 39.03 | nd |
| Bronchial epithelial cells | nd | nd | nd | 113.33 | nd |
| Squamous epithelial cells | nd | nd | nd | 15.00 | nd |
| IL16 | –75.16 | –48.01 | nd | nd | nd |
| IL2 | 12.90 | nd | nd | nd | nd |
| IL4 | 0.00 | nd | nd | nd | nd |
| IL6 | –62.94 | nd | nd | nd | nd |
| IL8 | –25.33 | nd | nd | nd | nd |
| IL10 | 0.00 | nd | nd | nd | nd |
| IL13 | 16.91 | nd | nd | nd | nd |
| IL 12p70 | 8.35 | nd | nd | nd | nd |
| IFNγ | 13.94 | nd | nd | nd | nd |
| TNFα | –5.76 | nd | nd | nd | nd |
| MPO | nd | –42.52 | nd | nd | nd |
| PGE2 | nd | –41.53 | nd | nd | nd |
| EN-RAGE | nd | –31.38 | nd | nd | nd |
| RAGE | nd | –69.91 | nd | nd | nd |
| MMP-9 | nd | –20.81 | nd | nd | nd |
| S100A8 | nd | 3.86 | nd | nd | nd |
| S100A9 | nd | 17.47 | nd | nd | nd |
| Galectin-3 | nd | –4.83 | nd | nd | nd |
| Uteroglobin/CC-10 | nd | –7.44 | nd | nd | nd |
| Lipids | | | | | |
| HDL | nd | nd | 2.47 | nd | nd |
| Growth factors (pg/mg protein) | | | | | |
| BDNF | nd | –84.91 | nd | nd | nd |
| Basic EGF | nd | –67.89 | nd | nd | nd |
| NGF | nd | –69.28 | nd | nd | nd |
| SCF | nd | –95.15 | nd | nd | nd |
| BMP-2 | nd | –88.36 | nd | nd | nd |
| IGF | nd | –39.59 | nd | nd | nd |
| PDGF-AA | nd | –62.79 | nd | nd | nd |
| TGF-α | nd | –33.99 | nd | nd | nd |
| EGF | nd | –53.37 | nd | nd | nd |
| PIGF | nd | –89.52 | nd | nd | nd |
| VEGF | nd | –49.95 | nd | nd | nd |
| Tissue injury and repair | | | | | |
| Serpine1/PAI-1 | nd | –21.21 | nd | nd | nd |

\(a\) Calculated in two ways. 1) Calculated by averaging the rate of change from baseline in individual subjects. [49]. 2) Calculate by using the mean (arithmetic mean, geometric mean, LS mean) or median of each marker at baseline and last day. [23,27,28,37,46–48,68].

\(b\) Bold is statistically significant.
e-cigarettes/HTPs. Of note, it has also recently been reported that HTPs showed reductions in quantitative risk estimates [70] and an absence of significant in vitro toxicological activity [71] compared to conventional cigarettes.

Despite these promising findings, the scientific literature about e-cigarettes and HTPs is diverse and specific consensus is lacking. In this review, a few biomarkers were not shown to be consistently changed, such as the sICAM1 [28], CRP [86,47], WBC [28], OxLDL [23], which could create difficulties in interpretation. Consequently some public health authorities have supported the use of e-cigarettes or HTPs only as a bridge to smoking cessation and warn about possible health effects, particularly among young and young adults [64]. More importantly it is still unknown whether e-cigarettes or HTPs have long-term effectiveness in reducing exposure to toxins compared to smoking combusted tobacco. Consequently, for the longer-term, little is known about the health effects of the use of e-cigarettes and HTPs, as relevant scientific evidence is currently not sufficient.

The results of our review suggest no major or consistent differences between e-cigarettes and HTPs. Levels of selected BOEs were similar in both groups, with similar reduction rates after switching from combusted tobacco. Regarding those biomarkers with a long half-life, only further studies with longer interventions and follow up periods are needed.

This systematic review is subject to some limitations. First, most clinical studies were manufacturer-funded studies, which could lead to publication bias. Second, since studies on BOBEs may require longer intervention periods, the number of reports was limited without the necessary follow up time to show changes in biological functions. Third, while the BOBEs employed in these studies may reflect processes on the pathway to smoking-related disease, their predictive and discriminative power has yet to be established so further studies such as long-term epidemiological studies are needed to show their relevance to tobacco related disease and the impact of HTP or e-cigarette use. We conclude that the current evidence supports the use of non-combustible smoking alternatives such as e-cigarettes and HTPs, which on the evidence presented in this review have been shown to improve levels of both BOEs and BOBEs. Although this may suggest plausible effects on the incidence of smoke-related disease, confirmatory data is not yet available, so this remains a fertile research area in the coming years.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.toxrep.2021.01.014.

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