Prevention and therapy of periodontal diseases and oral malodour

*Brush, rinse and cool*

van der Sluijs, E.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Dry-brushing: does it improve plaque removal? A secondary analysis

E. van der Sluijs
D.E. Slot
N.L. Hennequin-Hoenderdos
G.A. van der Weijden

International Journal of Dental Hygiene
2017 submitted (IDH-17-OA-1936)
Abstract

**Aim**
This paper is a secondary analysis comparing the effects on plaque removal of brushing with a dry toothbrush and brushing with a pre-wetted toothbrush.

**Methods**
The participants had been included in two previous experiments involving single-brushing exercises. The 46 non-dental participants were systemically healthy and ≥18 years of age. In the first experiment, the control intervention was brushing with a pre-wetted toothbrush, while during the second experiment it was brushing with a dry toothbrush. Both experiments scored plaque before and after the brushing exercises and assessed participants’ perception. The data of these two previous experiments were compared in this secondary analysis.

**Results**
Plaque levels before the brushing exercises were not significantly different (P = 0.054). Plaque score reduction following brushing with a dry toothbrush was 58%, while with a pre-wetted toothbrush it was 57%. The mean plaque index score reduction of 0.08 between a dry and a pre-wetted toothbrush was not significant (P = 0.096). Pre-wetting the participants’ toothbrush had no influence on the perception of toothbrush filament stiffness (P = 0.410) nor on the perception of cleaning capability (P = 0.449). In both experiments, brushing without dentifrice was judged to be unpleasant.

**Conclusion**
On average, following a two-minute brushing exercise, 57% or more dental plaque was removed. Dry brushing did not contribute significantly to toothbrush efficacy. The participants did not find that pre-wetting a toothbrush influenced the cleaning capability and filament stiffness.
Introduction

Teeth that are consistently surrounded by inflamed gingiva have a significantly higher risk of being lost (1). A determinant of the initiation of gingivitis (2) is supra-gingival plaque accumulation, which involves an established bacterial colonization on the dentition. Dental plaque control through routine oral hygiene is therefore important (3). It is well established that the toothbrush is effective in reducing levels of dental plaque on the surfaces of teeth (4), meaning that it plays an important role in the prevention of periodontal diseases (5). While brushing is a simple and effective means of removing dental plaque, there is clearly room for improvement (6). Oral hygiene is apparently a public and personal health issue, and improved hygiene could be expected to result in benefits in terms of periodontal disease and dental caries (6).

It is common practice to combine a toothbrush with dentifrice. Not only do many people like the resultant flavour and freshness, but it also provides the subjective impression of making the mouth feel clean (7). Dentifrice also adds a smooth feeling to tooth surfaces. In 1998, the concept of ‘dry brushing’ was introduced: brushing without dentifrice and a toothbrush not wetted with water (8). The purpose of this was to avoid the smooth perception of tooth surfaces being the results of reduced surface tension, as provided by surfactants of a dentifrice. In addition, a recent systematic review demonstrated that brushing with a dentifrice does not improve the efficacy of mechanical plaque removal (7). It is suggested that dry toothbrushing increases peoples’ ability to feel the bacterial biofilm, as well as to feel the difference in dental plaque on the tooth surfaces before and after brushing (9). Patients are instructed to start brushing on the lower lingual surfaces and to brush until all of the teeth feel clean. In a second variation of the experiment, dentifrice is added and the teeth are brushed once more. In a multicentre practice-based observational study, significant improvements in gingival bleeding were observed after six months of dry tooth brushing (8).

Currently, there is no high-quality research that has shown that dry brushing is indeed a more effective method. Plaque removal with a dry toothbrush has not been compared to that of a pre-wetted toothbrush with water. Recently, we published two (10,11) similar single-brushing exercises of which one included brushing with a pre-wetted (10) and the other brushing with a dry toothbrush (11). These experiments were performed under the same conditions with the same participants and the same examiners. Therefore, a secondary analysis could be performed using the available data of both previous experiments concerning the effectiveness of a dry toothbrush as compared to a pre-wetted toothbrush.
Materials and methods

The recommendations for strengthening the reporting were followed, as suggested by the guidelines outlined in Consolidated Standards of Reporting Trials (CONSORT) (12) and the checklist of the Template for Intervention Description and Replication (TIDieR) (13) were used.

Ethical procedures

Both original experiments approximated the Good Clinical Practice (CPMP/ICH/135/95) guidelines, in agreement with the ethical principles of the Declaration of Helsinki (2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO), as well as applicable local regulations. The experiments were approved by the medical ethical committee at Amsterdam Medical Centre (#2014_118) and were registered at the Dutch Trial Register (#NTR4604). The experiments took place at the Department of Periodontology of the Academic Centre for Dentistry of Amsterdam (ACTA) in the Netherlands in the period from June until September 2014. Before enrolment, all volunteers were given verbal and written information concerning the aim, rationale and duration of the original experimental aims. The investigator explained the details and potential risk involved. Prior to the study procedures, an informed consent form was signed by all eligible subjects that had agreed to participate, who were informed that they were free to withdraw at any time. Both previous experiments are separately published in the public domain as full scientific papers (10,11).

Recruitment and inclusion

The participants had been included in two previous experiments involving two single-brushing exercises (10, 11). They had been recruited from various universities and colleges in and around Amsterdam and had been screened by a dental hygienist (MPCL). To qualify for inclusion, the subjects were required to be ≥18 years old, right-handed brushers, classified as systemically healthy (as assessed by the medical questionnaire), periodontally healthy (scoring the DPSI ≤3 minus) (14) and retaining ≥5 teeth per quadrant. Excluded were those who presented the researchers with any of the following: an orthodontic appliance or a removable (partial) denture, overt caries, any pathological alterations of the oral mucosa, pregnancy or the use of medications within two weeks of the appointment. The latter included antibiotics or chronic use of non-steroidal anti-inflammatory drugs, although it excluded birth control pills.

Design

Both clinical experiments had a single-examiner blind design and used a split-mouth model in which contra-lateral quadrants were randomly assigned to treatment (15). There was one study coordinator (EVDS), who supervised the assignment of both brushing procedures. A priori sample-size calculations revealed that at least 40 participants were needed to be able to reject the null hypothesis that the response difference was zero with a probability (power) of 0.8 (10,11). Each participant received a unique subject identification
number that was used in both experiments. No stratification was applied. Randomization was performed within each experiment, using true random numbers that were generated by sampling and processing a source of atmospheric noise (16). The randomization code was kept in a sealed envelope in the investigator site file and was only accessible though the coordinator, who was therefore responsible for allocation concealment. To conceal the intervention from the examiners, the participants were instructed to not reveal their assignment in any way. Records of earlier examinations were not available to the examiner at any time.

**Outcomes**

In the first experiment, a pre-wetted toothbrush (10) was used and in the second experiment a dry toothbrush (11). The data of the control interventions of these two experiments were compared in this secondary analysis. The main study parameter was the level of dental plaque scored according to the criteria of the modified Plaque Index (PI) (17,18). Throughout the experiments, examinations were sequentially carried out by one and the same experienced trained examiner (NLHH) under the same conditions. The secondary outcome was an evaluation of the self-perception of the participants as derived with the aid of a visual analogue scale (VAS) (19). After the assessment, the participant completed the questionnaire, which has been designed to evaluate their opinion and their perception concerning the 48-hour non-brushing period, the toothbrush and the procedure used. They were requested to place a vertical mark on a 10-cm long uncalibrated line (0-10). The left extreme represented the negative (score 0), whereas the right extreme represented the positive (score 10). The investigator measured the distance along the line from the left extreme to the marking made by the participant.

**Study procedure**

Text messages (Short Message Service) were sent to remind each participant before the visits concerning the study procedures and appointments. A 48-hour non-brushing period was instigated in order to accumulate an adequate amount of plaque to assess the incremental effect on plaque scores of both single-brushing exercises. Firstly, a full-mouth dental plaque score was performed. Before the brushing exercise of each visit, a short verbal instruction was given to the participant. The supervised brushing procedure was performed according to the bass-method (20), using a new Oral-B indicator P35 soft toothbrush without dentifrice for each experiment. The brushing order was carefully standardized and controlled, including guidance through the randomized set of contra-lateral quadrants. Each buccal or lingual side of a quadrant was brushed for 15 s, meaning that a total of 30 s was spent per quadrant, as controlled through the use of a timer. No mirror was available during the brushing procedures, and the participants were able to expectorate if necessary.

**Statistical analysis**

The data of both experiments were merged and allocated. Only participants that completed both experiments were taken into account for this secondary analysis (per protocol
Figure 1 Flow Chart depicting subject enrollment and measurement (12).

Recruitment

Informed consent + Screening (DPSI scores)

Assessed for eligibility [N=50]

Excluded (N=2):
  - Overt caries [N=1]
  - Periodontitis [N=1]

Experiment 1 (10)

Full mouth S&L plaque index

Supervised brushing using a prewetted toothbrush [N=48]

Full mouth S&L plaque index and questionnaire I

No study period

Experiment 2 (11)

Full mouth S&L plaque index

Supervised brushing using a dry toothbrush [N=46]

Full mouth S&L plaque index and questionnaire II

Analysis

Secondary analysis [N=46]
analysis). The data of two contra-lateral quadrants that were used in both experiments as control sites were extracted. The statistical analysis was performed using a statistical package (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). The primary parameter was the overall dental plaque score for which the mean and standard deviation of all readings were calculated for each participant. In addition, the mean incremental difference and proportional change were computed. Dental plaque scores were analysed by each regimen. The mean scores and standard deviation regarding the VAS questions were calculated. A parametric statistical approach was used through a paired sample T-test. Values of p≤0.05 were accepted as statistically significant.

Results

Participants
In Figure 1, the study design flow of both experiments is illustrated, including the same participants and performed by the same research group. Regarding eligibility, a total of 50 participants were assessed and, accounting for potential drop-outs, 48 participants were included for the first experiment (10). From the same study population, 46 participants also willingly entered the second experiment (11). The two other participants lost to the follow-up experienced scheduling conflicts, which was interpreted as drop-outs unrelated to the interventions. Therefore, a total of 46 participants could be included for this secondary analysis. The mean age was 22.5 (2.86), with a range of 20 to 34 years. More female participants were included in these clinical experiments (N=27; 59%) than men (N=19; 41%). No adverse events were reported.

Primary outcome
Table 1 shows the mean and standard deviation of the overall dental plaque scores of brushing with a dry toothbrush and a pre-wetted toothbrush. For pre-brushing, the mean plaque scores were not significantly different (P = 0.054). With regard to post-brushing, the mean plaque scores also did not differ significantly (P = 0.713). A mean plaque score reduction of 0.75 (0.33) with the pre-wetted toothbrush was found, while the dry toothbrush showed a reduction of 0.76 (0.38). The numerical incremental mean difference of 0.08 between the regimens was not statistically significant (P = 0.096).

Analysis of covariance
Pre-brushing plaque scores were close to being significantly different (P = 0.054), which could have affected the primary outcome. Therefore, in addition, an analysis of covariance (ANCOVA) was performed. For this analysis, the pre-brushing scores were used as a covariate. A statistical difference was not detected for either the post-brushing scores (P = 0.269) or the incremental differences (P = 0.269).
Secondary outcome

A questionnaire regarding the participants’ perception was completed in both experiments. In total, four questions were identical and could be analysed in this secondary analysis (Table 2). The specific instruction to refrain from oral hygiene for 48 hours was experienced as most uncomfortable experience in both experiments (2.57 and 2.59 respectively). Brushing without dentifrice was also judged to be unpleasant irrespective of the fact whether the toothbrush was dry (3.21) or pre-wetted (3.01). Toothbrush filament stiffness was judged to be in the middle between soft and rigid (4.87) for the pre-wetted toothbrush and 3.21 for a dry toothbrush (P = 0.410). The perception of cleaning capability received mean VAS scores of 6.04 and 6.29. None of the outcomes to the questions indicated a significant difference between dry brushing or brushing with a pre-wetted toothbrush.

<table>
<thead>
<tr>
<th>N=46</th>
<th>Pre-wetted toothbrush</th>
<th>Dry toothbrush</th>
<th>P-value*</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-brushing</td>
<td>1.65 (0.34)</td>
<td>1.74 (0.30)</td>
<td>0.054</td>
<td></td>
</tr>
<tr>
<td>Post-brushing</td>
<td>0.75 (0.33)</td>
<td>0.76 (0.38)</td>
<td>0.713</td>
<td>0.269</td>
</tr>
<tr>
<td>Incremental difference</td>
<td>0.90 (0.20)</td>
<td>0.98 (0.31)</td>
<td>0.096</td>
<td>0.269</td>
</tr>
<tr>
<td>% Reduction</td>
<td>57%</td>
<td>58%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Paired sample T-test
** Univariate analysis of variance with pre-brushing scores as covariance

Table 1 Overall dental plaque scores on pre- and post-brushing presented by mean and SD (standard deviation) in relation to the use of a dry toothbrush or pre-wetted toothbrush.

Table 2 Questionnaire related to the self-perception of the participants using a Visual Analogue Scale (VAS), presented as mean and standard deviation (SD) with negative extremes on the left and positive extremes on the right (from 0 to 10).

<table>
<thead>
<tr>
<th>N=46</th>
<th>Extreme From</th>
<th>To</th>
<th>Pre-wetted toothbrush</th>
<th>Dry toothbrush</th>
<th>Statistical analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>What do you think of refraining from 48 hours before the appointment?</td>
<td>Very uncomfortable</td>
<td>Very comfortable</td>
<td>2.57 [1.57]</td>
<td>2.59 [1.73]</td>
</tr>
<tr>
<td>2</td>
<td>I think the toothbrush is cleaning my teeth well enough...</td>
<td>Totally not agree</td>
<td>Fully agree</td>
<td>6.04 [2.05]</td>
<td>6.29 [1.93]</td>
</tr>
<tr>
<td>3</td>
<td>I think that the stiffness of the toothbrush filament is...</td>
<td>Very soft</td>
<td>Very rigid</td>
<td>4.87 [1.65]</td>
<td>3.21 [2.45]</td>
</tr>
<tr>
<td>4</td>
<td>Brushing without toothpaste was...</td>
<td>Very unpleasant</td>
<td>Very pleasant</td>
<td>3.01 [2.45]</td>
<td>3.21 [2.33]</td>
</tr>
</tbody>
</table>

* Paired sample T-test
Discussion

Approximately twenty years ago, it was suggested that brushing without dentifrice allows the patient to more distinctly feel the layer of dental plaque before and after brushing. This was considered not to be the case with a dentifrice due to associated flavour and wetting agents (8). By the use of a secondary analysis, the aim of this paper is to evaluate the effectiveness of a dry toothbrush as compared to a pre-wetted toothbrush on plaque removal. The overall reduction in dental plaque scores was at least 57% following a two-minute brushing exercise (pre-wetted toothbrush 57%, dry toothbrush 58%). Consequently, dry brushing did not contribute significantly to toothbrush efficacy. Based on the results of this secondary data analysis, the recommendation to use a dry toothbrush is not supported by evidence. Pre-wetting a toothbrush neither improved nor reduced plaque removal efficacy. The minimal 57% overall reduction in dental plaque scores found in the present analysis was higher than the 42% reduction established as the average effect that can be expected from a brushing exercise (21). This implies that the participants of the present experiments were above-average brushes. There are almost twice as effective as the average participant of those studies reporting efficacy according to Quigley and Hein plaque scores (22), who on average achieved a 30% reduction (21). Supervised brushing may have improved plaque score reduction in the current experiment. Supervision was performed to ensure that the study procedures including brushing duration were according to the protocol (23).

The concept of ‘dry brushing’ was introduced based on a multi-centre observational study (8). However, this study however lacks a control group. Furthermore, for evaluating the effectiveness of interventions, a randomised controlled trial (RCT) would be more appropriate, as RCTs are generally placed at the top of the research hierarchy when considering original experimental studies. This secondary analysis used the data of two previous experiments and found a larger effect in overall reduction in dental plaque scores compared to the dental plaque score reduction as shown as the average effect of a single brushing exercise (22). The advantage of the larger effect size is that it is possible to detect a difference between interventions in smaller sample numbers, whereas a smaller effect size would require larger sample sizes (24). Subsequently this secondary analysis shows that dry brushing does not contribute to plaque-removing efficacy.

Study design used

Both original experiments were based on a split-mouth randomization (15). The participants with a dry toothbrush brushed as a control intervention in one experiment (11), while they brushed with a pre-wetted toothbrush in the other experiments (10). In each experiment, the population brushed one set of the two randomized contra-lateral quadrants (either the 1st and 3rd or the 2nd and 4th quadrant), as the control treatment was similar for each participant quadrants in the two experiments. Because this was a secondary analysis, the interventions (dry or pre-wetted) were not randomized. Theoretically, the examiner could have known which intervention was performed in each experiment, which may have influenced the judgement. However, if existent, the impact of this influence is
presumably small because in each experiment another intervention was also performed in the opposing quadrants assigned for the test (e.g. pre-rinsing with water (10) and a specific brushing sequence (11)). The examiner was blinded for the two randomized interventions (test and control). In fact for in the present analysis the randomization of dry and wet brushing was not performed. The decision to compare the data on the use of a dry or a wet toothbrush was made after both previous experiments had been published and the aims of both papers were different from the present analysis. Since the conceptual idea of this secondary analysis evaluating a dry toothbrush to a pre-wetted toothbrush on plaque removal was developed after analysing the data from the previous two original experiments, the lack of randomization has most likely also not influenced the examiner.

**Several ‘dry brushing’ definitions**

Dry tooth brushing was already reported over thirty years ago (25) and was described as brushing ‘without the use of a dentifrice’. In those days, this was suggested to minimize the classroom disruption and the length of time associated with daily brushing in a kindergarten classroom. In addition, other studies most likely reported the use of a ‘dry toothbrush’ as brushing without dentifrice (26,27), although this was mainly for practical or research purposes rather than the intention of improving plaque removal. The original O’Herir and Suvan 1998 (8) study suggested that dry tooth brushing was both without dentifrice and without water. A recent systematic review establishing the efficacy of brushing with and without a dentifrice for dental plaque removal (7) did not explore whether or not the brush in the underlying papers was pre-wetted. The current study is the first of its kind to specifically evaluate the use of a new toothbrush wetted with water before its use in the mouth compared to an identical new and dry toothbrush. Considering that there are more options on how a ‘dry toothbrush’ can be interpreted in the literature, it is advisable that studies should report interventions in greater detail. For the ultimate reliability and completeness of interventions, the Template for Intervention Description and Replication (TIDieR) checklist is developed (13). The TIDieR checklist and guide should improve the reporting of interventions and facilitate authors structuring accounts of their interventions, reviewers and editors assessing the descriptions and readers using the information. The use of this checklist in addition to the CONSORT (12) is a recommendation for the reporting of research in the future.

**Participant’s perceptions**

Another finding of the current study is that participants do not appear to like brushing without a dentifrice, irrespective of whether or not the brush is pre-wetted. As early as 1960, Dudding et al. (29) concluded that almost 50% of people would not brush their teeth if they could not use a dentifrice. These patient-reported outcomes have become more important and meaningful over time (30), as these directly reflect how the participant feels or functions in relation to his/her health condition. Therefore, in the present studies, the self-perception of the participants was assessed by using the VAS. The fourth question of Table 2 illustrates that, in both original experiments, the participants brushing without dentifrice score on the negative side of the VAS. It was judged on a scale of zero to ten.
where ‘5’ is considered as neutral, with mean scores of 3.21 and 3.01 the perception on the left (negative) side of the perception. Traditionally, dentifrices have played an important role in improving oral freshness and combatting tooth discoloration (7,31,32), and this may subsequently stimulate daily oral self-care. As suggested by Valkenburg et. al (2016) (7), future research into the psychosocial factors that might influence attitude towards and performance in oral hygiene is needed (33). Dentifrices are also the most effective fluoride carriers, and their contribution to the prevention of caries is well established (34). They may also contain chemical agents for the treatment of malodour, staining, caries, gingivitis, dental plaque, dental calculus, demineralization and dentinal hypersensitivity (32,35). The present study did not use dentifrice; a direction for further research may be the use of a dry or pre-wetted toothbrush in combination with dentifrice, as wetting may improve the availability of chemotherapeutic agents from the paste.

**Secondary analysis**

Currently, a secondary analysis is a rather uncommon method of analysis in dental research. A combination of data sharing and secondary analysis of archived data can contribute to the advancement of knowledge and greater transparency in scientific inquiry (36, 37). The analysis of existing data is a cost-efficient way to make full use of data that have already been collected. This is done in order to both address new research questions and provide a considerate assessment of the primary results of the original study (36). Until recently, there was no guideline for systematic, transparent and complete reporting of secondary data. However, a Standardized Reporting of Secondary Data Analyses (STROSA) statement has been published in the German language. This includes 27 items regarding methodological quality and supports authors and readers in critical appraisal (37,38). This checklist was translated by the authors of the present paper and adapted for the present study. It is available as appendix (S1).

**Limitations**

- Earlier research has suggested that, without dentifrice, people brush for much longer and more evenly around the mouth (9). In order to reduce the number of variables, in the two original experiments, time was restricted to 30 second per quadrant, and participants were supervised and guided through the brushing procedure. Therefore, the possible stimulating effect of brushing without dentifrice could not be evaluated.
- The experiments were well controlled and performed in a clinical setting. Participants possibly could have been more at ease while brushing at home and may have behaved accordingly differently.
- Regardless of whether it is wet or dry, a toothbrush is also moistened with the saliva of the user as soon as it is used in the oral cavity.
Conclusion

On average, a two-minute brushing exercise removed at least 57% of dental plaque. Dry brushing did not contribute significantly to toothbrush efficacy. The participants did not find that pre-wetting a toothbrush influenced filament stiffness and the cleaning capability. They also perceived brushing without dentifrice to be uncomfortable.

Conflict of interest and source of funding statement

Both experiments (10, 11) were sponsored through an unrestricted educational grant from the Procter & Gamble Company Clinical Investigations - Oral Care. The manual toothbrushes were also provided free of charge by Procter & Gamble.

The authors declare that they have no conflicts of interest. Van der Weijden, Slot and their research team at ACTA have previously received either external advisor fees, lecturer fees or research grants from toothbrush and dentifrice manufacturers. Those manufacturers included Braun, Colgate, Dental, GABA, Lactona, Meda Pharma, Oral-B, Procter & Gamble, Sara Lee, Sunstar and Unilever.

Authors contributions
Conception or design of the study: EVDS, DES, NLHH, GAW
Analysis and/or interpretation of the data: EVDS, DES, GAW
Drafted the manuscript: EVDS, DES
Critically revised the manuscript: DES, NLHH, GAW
All authors gave their final approval and agreed to be accountable for all aspects of the work
**Appendix S1** Standardized Reporting Of Secondary data Analyses (STROSA) statement regarding methodological quality. This checklist is translated by the authors of the present paper and filled in [37-38].

<table>
<thead>
<tr>
<th>Number</th>
<th>Criteria</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Title and abstract</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Keywords</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><strong>Introduction</strong></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Background and Rationale</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Objectives</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><strong>Methods</strong></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Study design</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>Data source</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>Legal</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>Data protection</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>Flow chart</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>Selection criteria</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>Analysis</td>
<td>9</td>
</tr>
<tr>
<td>12</td>
<td>Variables</td>
<td>7</td>
</tr>
<tr>
<td>13</td>
<td>Study size</td>
<td>9</td>
</tr>
<tr>
<td>14</td>
<td>Statistical methods</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number</th>
<th>Criteria</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Selection of the study population</td>
<td>6, 7</td>
</tr>
<tr>
<td>16</td>
<td>Descriptive results</td>
<td>9</td>
</tr>
<tr>
<td>17</td>
<td>Main results</td>
<td>9, 10</td>
</tr>
<tr>
<td>18</td>
<td>Further results</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td><strong>Discussion</strong></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Main results</td>
<td>11</td>
</tr>
<tr>
<td>20</td>
<td>Internal validity and risk distortion</td>
<td>11, 12</td>
</tr>
<tr>
<td>21</td>
<td>Strengths and weaknesses</td>
<td>11, 12</td>
</tr>
<tr>
<td>22</td>
<td>Interpretation</td>
<td>12</td>
</tr>
<tr>
<td>23</td>
<td>External validity</td>
<td>11, 12</td>
</tr>
<tr>
<td></td>
<td><strong>Conclusion</strong></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Conclusion</td>
<td>14</td>
</tr>
<tr>
<td>25</td>
<td>Financing/ sponsor</td>
<td>1, 14</td>
</tr>
<tr>
<td>26</td>
<td>Authors’ responsibility</td>
<td>Online</td>
</tr>
<tr>
<td>27</td>
<td>Conflict of interest</td>
<td>1</td>
</tr>
</tbody>
</table>
References