Oral Treatment With Bisphosphonates of Osteoporosis Does Not Increase the Risk of Severe Gastrointestinal Side Effects: A Meta-Analysis of Randomized Controlled Trials

Zsuzsa Réka Dömötör1,2, Nóra Vörhendi2, Lilla Hanáč2, Péter Hegyi2, Szabolcs Kiss2,3, Endre Csiki2, Lajos Szakó2, Andrea Párniczky2 and Bálint Erőss2*

1 Faculty of Medicine, University of Medicine, Pharmacy, Science and Technology of Targu Mures, Targu Mures, Romania, 2 Institute for Translational Medicine, University of Pécs, Medical School, Pécs, Hungary, 3 Doctoral School of Clinical Medicine, University of Szeged, Szeged, Hungary

Introduction: Bisphosphonates (BPs) are first-line therapy for osteoporosis. Adherence is usually low in chronic, asymptomatic diseases, but gastrointestinal (GI) side-effects can also contribute to low adherence in BP therapy and may necessitate a review by a gastroenterologist with or without gastroscopy.

Aims: Our meta-analysis aims to determine the risk of severe GI adverse events due to oral BP therapy in osteoporotic patients.

Methods: A systematic search was conducted in three databases up to September 2020 for randomized controlled trials (RCTs) detailing GI adverse events in adults with osteoporosis on BP compared to placebo. Risk ratios (RRs) with 95% confidence intervals (CI) were calculated for non-severe and severe adverse events indicating endoscopic procedure with the random-effects model. Statistical heterogeneity was assessed using chi² and I² statistics.

Results: Forty-two RCTs with 39,047 patients with 9,999 non-severe and 1,503 severe GI adverse events were included. The incidence of non-severe and severe adverse events ranged between 0.3–54.9 and 0–10.3%, respectively. There was no difference between BP and control groups in terms of the risk of non-severe or severe side effects: RR=1.05 (CI: 0.98–1.12), I² = 48.1%, and RR=1.01 (CI: 0.92–1.12), I² = 0.0%, respectively. Subgroup analysis of the most commonly used BP, once-weekly alendronate 70 mg, revealed an association between bisphosphonates and the risk of non-severe GI adverse events, RR=1.16 (CI: 1.00–1.36), I² = 40.7%, while the risk of severe GI side effects was not increased in this subgroup, RR=1.20 (CI: 0.83–1.74), I² = 0.0%.

Conclusion: Our results show that bisphosphonates do not increase the risk of severe GI adverse events. However, the marked variability of the screening for side effects in the
INTRODUCTION

Osteoporosis is a systemic bone disease with low bone mineral density and poor bone microarchitecture which leads to an increased risk of fracture (1). According to the most recent Osteoporosis Guideline, oral bisphosphonates (BPs) are one of the most commonly used therapeutic agents in patients with osteoporosis (2). Adherence is usually low in chronic, asymptomatic diseases, but gastrointestinal (GI) side-effects can also contribute to low adherence in BP therapy and may necessitate a review by a gastroenterologist with or without gastroscopy (3, 4). A cross-sectional patient survey showed that these GI side effects account for 40% of all discontinuation (5). Most commonly these are reported in the foregut, including heartburn, nausea, vomiting, epigastric pain, esophagitis, gastric ulcer, dyspepsia, and GI bleeding (6).

While the efficacy of BPs is out of debate, previous systematic reviews and meta-analysis investigating the tolerability of bisphosphonates did not determine the risk of severe and non-severe GI side effects of oral bisphosphonates.

There have been studies investigating the bisphosphonates-caused mucosal damage of the upper GI tract since it became an established drug in the treatment of osteoporosis (7–9). None of the previous meta-analyses in this topic focused on the risks of severe GI side effects. We aimed to differentiate between the mild and severe side effects and determine the risks of these side effects in case of all commonly used oral bisphosphonates for osteoporosis.

METHODS

Protocol

Our meta-analysis and systematic review is reported using the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) (10). The project was registered in October 2019 on PROSPERO the registration number is CRD42020147522.

Eligibility Criteria

Our scientific question, using the population-intervention-control-outcomes (PICO) framework was: (P) adult patients with primary osteoporosis, (I) oral bisphosphonates, (C) placebo or vitamin D or calcium, but no other medication for osteoporosis, and (O) severe and non-severe GI adverse events. Articles were included if they provided relevant information about any drug-induced GI adverse event. Only full-text articles and randomized controlled trials (RCTs) were included.

Search Strategy

A systematic search was conducted in 3 databases, MEDLINE (via PubMed), Embase, and Cochrane Central Register of Controlled Trials from inception to 6th September 2020. Keywords for the computer-aided search were ((diphosphonate OR bisphosphonate OR etidron* OR clodron* OR tiludron* OR pamidron* OR neridron* OR alendron* OR ibandron* OR risedron* OR zoledron*) AND (gastrointestinal OR digestive OR “alimentary tract” OR esophageal OR oesophageal OR esophageus OR oesophageus OR gastric OR stomach OR antrum OR antral OR pylorus OR pyloric OR gastroduodenal OR duodenal OR duodenum OR bowel OR intestine OR intestinal OR colon OR colonic OR viscus OR visceral OR abdomen OR abdominal)) with the “Human” filter applied, but without other restrictions to language or other features.

Study Selection

Records were managed by EndNote X9 (Clarivate Analytics, Philadelphia, PA, USA). After the exclusion of duplicates, the remaining records were screened by title, abstract, and full-text independently by two review authors (ZRD, NV). Additional articles were manually searched and identified from the reference lists of eligible primary studies. Disagreements were resolved by consensus or by the involvement of the senior review author (BE).

Data Extraction

Numeric data were extracted by two review authors (ZRD, NV) and manually populated onto a purpose-designed Excel 2019 sheet (Office 365, Microsoft, Redmond, WA, USA). Data were collected from each paper on the year of publication, study design, country, the number of randomized patients, and baseline patient characteristics (age, sex, race, history of GI, body mass index, tobacco, alcohol, and caffeine usage in both groups). Most importantly, data were collected on the non-severe and severe GI adverse events. To ensure that results of the included studies were uniformly assessed as intention-to-treat protocol, in cases of per-protocol analyses the missing data were imputed, missing subjects were regarded as not having adverse events. Adverse events reported in the original studies were categorized by the review authors following the U.S. Food and Drug Administration criteria (11), detailed in Supplementary Table 1. Data on type of the bisphosphonate used as treatment and the control treatment, dosage, duration, route, and schedule of administration, follow-up period were also extracted. Disagreements were resolved by consensus or by the involvement of the senior reviewer (BE).
Statistical Analysis
Risk ratios (RRs) with 95% confidence intervals (CI) were calculated for non-severe and severe adverse events with the random-effect model by DerSimonian-Laird (12). Subgroup analyses were performed for the different bisphosphonates (alendronate, risedronate, etidronate, pamidronate, ibandronate), the different dosage of the bisphosphonates and the duration of administration. Statistical heterogeneity was assessed using chi² and I² statistics. Statistical heterogeneity was assessed using Cochrane’s Q and the I² statistics. According to the Cochrane Handbook for Systematic Reviews of Interventions (13), heterogeneity could be interpreted as moderate between 30 and 60%, as substantial between 50 and 90% and as considerable above 75%. The presence of publication bias was assessed by visual inspection of the funnel plots and Egger’s test (14), and the effect of publication bias was evaluated by the trim-and-fill method (15). A significant test result from Egger’s test (p<0.1) indicates the presence of bias. We also performed Trial Sequential Analysis (TSA) for the primary outcomes to evaluate whether further randomized trials are futile to show or discard the anticipated intervention effect. Statistical analyses were performed with Stata 16 (Stata Corp, College Station, TX, USA) and trial sequential analysis program version 0.9 beta (available from www.ctu.dk/tsa).

Risk of Bias Assessment
The quality assessment was done at the study level and then summarized. We used the revised Cochrane Collaboration’s risk-of-bias tool for randomized trials (16) for methodological quality assessment of the individual studies included in our meta-analysis. The risk of bias was assessed independently by three investigators (ZRD, NV, EC). Disagreements were resolved by consensus and the involvement of the corresponding author.

Assessment of the Grade of Evidence
The GRADE approach was used to assess the certainty of evidence regarding the outcomes. GRADE stands for Grades of Recommendation Assessment, Development, and Evaluation (17).

GRADE was assessed independently by two investigators (ZRD, EC). Disagreements were resolved by consensus and with the involvement of the corresponding author.

RESULTS
Results of the Selection Process
Our search strategy initially identified 8,392 studies, out of those 42 relevant articles were included in the qualitative and 39 in the quantitative synthesis of this meta-analysis. The study selection process is shown in Figure 1. The summary of the characteristics of the studies included in our analysis is shown in Table 1. In case of six studies missing data for intention-to-treat analysis were imputed (27, 40, 43, 44, 55, 56).

Adverse Events
The forty-two RCTs included 39,047 patients with 9,999 non-severe and 1,503 severe GI adverse events. The incidence of non-severe and severe adverse events ranged between 0.3–54.9, and 0–10.3%, respectively. The most common non-severe adverse events were nausea, vomiting, dyspepsia, and abdominal pain, while the vast majority of the severe side effects occurred in the esophagus (Supplementary Table 2).

Intervention: Bisphosphonates
Our meta-analysis included data from studies with four bisphosphonates: alendronate, risedronate, etidronate, and ibandronate. One study with pamidronate was included in the qualitative synthesis. All studies used orally administered bisphosphonates. Dosages and other details are shown in Table 1.

Results of Statistical Analysis
Bisphosphate Use Is Not Associated With the Risk of Non-Severe Adverse Events
The analysis for non-severe GI adverse events included 39 studies in the quantitative analysis. The number of overall non-severe GI adverse events were 5,486 in the bisphosphonate group and 4,450 in the control group. Compared against controls, the bisphosphonate use was not associated with the risk of non-severe side effects, RR=1.05, CI: 0.98–1.12, p=0.207 the heterogeneity was moderate: I² = 48.1%, p=0.001 (Figure 2).

Among non-serious GI adverse events alendronate, risedronate and ibandronate had 27, ten, and one studies included, respectively. Subgroup analysis for the three different bisphosphonates did not show an association with the risk of non-severe side effects (Supplementary Figure 1).

Bisphosphate Use Is Not Associated With Increased Risk of Severe Adverse Events
The number of overall severe GI adverse events were 874 in the bisphosphonate group and 629 in the control group.

The bisphosphonate use was not associated with the risk of severe side effects, compared against controls, RR=1.01, CI: 0.92–1.12, p=0.776; there was no significant heterogeneity: I² = 0.0%, p=0.979 (Figure 3).

Among serious upper GI events alendronate, risedronate, ibandronate, and etidronate had 24, ten, one, and one studies included, respectively. Subgroup analysis for the three different bisphosphonates did not show an association with the risk of non-severe side effects (Supplementary Figure 2).

Subgroup Analysis of Trials With the Primary Outcome of GI Tolerability of BP Therapy Showed No Increased Risk of GI Adverse Events
The number of overall non-severe GI adverse events was 1,956 in the bisphosphonate group and 1,912 in the control group.

Compared to controls, the BP use was not associated with the risk of non-severe side effects, RR=1.16, CI: 0.85–1.57, p=0.356, with considerable heterogeneity: I² = 75.0%, p=0.001 (Figure 4A).

The number of overall severe GI adverse events was 276 in the bisphosphonate group and 248 in the control group. The BP use
was not associated with the risk of severe side effects, compared against controls, RR=1.06, CI: 0.89–1.25, p=0.529; there was no significant heterogeneity: $I^2 = 0.0\%$, p=0.608 (Figure 4B).

**Long-Term Administration of Bisphosphonate Is Not Associated With Increased Risk of Side Effects**

In 15 eligible articles, there was no association between BP use and the risk of non-severe side effects in the subgroup of studies, where the treatment was at least 24 months, RR=1.00, CI: 0.93–1.08, p=0.983, heterogeneity was moderate: $I^2 = 53.9\%$, p=0.007 (Supplementary Figure 3).

In 14 eligible articles, there was no association with the risk of severe side effects in the subgroup of studies where the treatment was at least 24 months, RR=1.00, CI: 0.90–1.11, p=0.944 there was no significant heterogeneity: $I^2 = 0.0\%$, p=0.858 (Supplementary Figure 4).

**Non-Severe and Severe Adverse Events in the Context of the Most Commonly Used BP Therapies**

Subgroup analysis of the most commonly used BP, alendronate 10 mg/day or once-weekly alendronate 70 mg, revealed an increased risk of non-severe GI adverse events compared against controls, RR=1.16, CI: 1.00–1.36, p=0.056, with moderate heterogeneity: $I^2 = 40.7\%$, p=0.031, while the risk of severe adverse events was not increased in this subgroup RR=1.20, CI: 0.83–1.74, p=0.328, without significant heterogeneity: $I^2 = 0.0\%$, p=0.897 (Supplementary Figures 5, 6).

**Trial Sequential Analysis**

In case of non-severe adverse events, the cumulative z-curve crosses the futility boundary, which provided evidence indicating that no significant difference exists between the groups, and thus, further trials are not required (Supplementary Figure 7). In case of severe adverse events, the same conclusion could not be drawn as the acquired information size was substantially below the required information size (0.76%) by performing the TSA.

**Risk of Bias Assessment**

According to the Revised Cochrane Risk of Bias Assessment tool for RCTs the risk of bias was low in 25 studies, there were some concerns in 11, and high in six studies. Nearly all studies carried an unknown risk of reporting bias due to the lack of pre-study protocols. The detailed results of the assessment are shown in Supplementary Table 2.
| Author, year/ reference no. | Region/country | N° of centers | N° of patients in BP/control group (mean years) | Female ratio | Active substance Dosage (mg) | Control group | Follow-up (months) | Patients with pre-existing and/or previous GI diseases excluded | Incidence of nonsevere AE | Incidence of severe AE |
|-----------------------------|----------------|---------------|-----------------------------------------------|--------------|----------------------------|---------------|------------------|---------------------------------------------------|--------------------------|------------------------|
| Adachi et al., 2009 (18)    | Canada and Colombia | 34            | 291/147                                       | 100%         | Alendronate 10              | Placebo       | 3                | No                                  | 5.5%                     | 0.5%                   |
| Ascott-Evans 2003 (19)      | Argentina, Australia, Brazil, New Zealand, Africa, Europe | 18            | 95/49                                         | 100%         | Alendronate 10              | Placebo       | 12               | No                                  | 14.6%                    | 0.0%                   |
| Bauer et al., 2000 (20)     | United States of America | 11           | 3,236/3,223                                   | 100%         | Alendronate 5               | Placebo       | 45               | Yes                                 | 54.7%                    | 7.5%                   |
| Bell et al., 2002 (21)      | United States of America | 8            | 33/32                                         | 100%         | Alendronate 10, 5, after 2 years 10 | Placebo       | 24               | Yes                                 | 33.8%                    | 3.1%                   |
| Black et al., 1996 (22)     | United States of America | 11           | 1,022/1,005                                   | 100%         | Alendronate 5               | Placebo       | 24               | Yes                                 | 42.8%                    | 5.0%                   |
| Bone et al., 2000 (23)      | United States of America | 18           | 92/50                                         | 100%         | Alendronate 10              | Placebo       | 24               | Yes                                 | 18.3%                    | 0.0%                   |
| Boonen et al., 2009 (24)    | Eastern and Western Europe, Lebanon, Australia, USA | 24           | 191/93                                        | 0%           | Risedronate 35              | Placebo       | 24               | No                                  | 10.9%                    | 3.5%                   |
| Chesnut et al., 2004 (25)   | Canada, United States of America, Europe | 73           | 975/977                                       | 100%         | Ibandronate 2.5             | Placebo       | 36               | No                                  | 20.8%                    | 10.3%                  |
| Clemmesen et al., 1997 (26) | Denmark, Belgium | 2            | 44/44                                         | 100%         | Risedronate 2.5             | Placebo       | 36               | Nil                                 | 1.1%                     | 6.8%                   |
| Cryer et al., 2005/1 (27)   | United States of America | 51           | 224/230                                       | 100%         | Alendronate 70              | Placebo       | 6                | Yes                                 | 19.8%                    | 1.3%                   |
| Cryer et al., 2005/2 (28)   | United States of America | 48           | 224/226                                       | 92.5%        | Alendronate 70              | Placebo       | 3                | Yes                                 | 12.4%                    | 0.0%                   |
| Cummings et al., 1998 (29) | United States of America | 11           | 2,214/2,218                                   | 100%         | Alendronate 5, after 2 years 10 | Placebo       | 24               | Yes                                 | 23.6%                    | 0.8%                   |
| Downs et al., 2000 (30)     | United States of America | 24           | 118/58                                        | 100%         | Alendronate 10              | Placebo       | 12               | Only esophageal motility disorders | 18.8%                    | 0.0%                   |
| Eisman et al., 2004 (31)    | Europe, Australia, USA, Africa, Asia-Pacific | 44           | 225/224                                       | 94.2%        | Alendronate 70              | Placebo       | 3                | No                                  | 10.0%                    | 1.1%                   |
| Felsenberg et al., 1998 (32)| Argentina, Australia, Canada, Colombia, Europe | 62           | 219/223                                       | 100%         | Alendronate 10              | Placebo       | 12               | Yes                                 | 29.2%                    | 2.5%                   |
| Fogelman et al., 2000 (33)  | UK, France, Netherlands, Belgium, Germany | 13           | 184/180                                       | 100%         | Risedronate 2.5             | Placebo       | 36               | No                                  | 23.4%                    | 6.0%                   |
| Greenspan et al., 2002 (34) | United States of America | 48           | 224/226                                       | 92.4%        | Alendronate 70              | Placebo       | 3                | Only esophageal motility disorders | 14.0%                    | 1.3%                   |
| Harris et al., 1999 (35)    | North America | 110          | 813/815                                       | 100%         | Risedronate 5               | Placebo       | 36               | No                                  | 24.4%                    | 5.7%                   |
| Hosking et al., 2003 (36)   | Europe and Brazil | 38           | 222/108                                       | 100%         | Risedronate 5               | Placebo       | 12               | Only esophageal motility disorders | 0.3%                     | 1.2%                   |
| Ilter et al., 2006 (37)     | Turkey | 1            | 219/108                                       | 100%         | Alendronate 70              | Placebo       | 3                | No                                  | 0.6%                     | 0.9%                   |

(Continued)
| Author, year/reference no. | Region/country | N° of centers | N° of patients in BP/control group (mean years) | Female ratio | Active substance | Dosage (mg) | Control group | Follow-up (months) | Patients with pre-existing and/or previous GI diseases excluded | Incidence of nonsevere AE | Incidence of severe AE |
|---------------------------|----------------|---------------|-----------------------------------------------|--------------|-----------------|------------|---------------|-----------------|-----------------------------------------------|-------------------------|-------------------------|
| Iwamoto et al., 2001 (38) | Japan          | 1             | 25/24                                         | 64.3/66      | 100% Etidronate | 200§       | Calcium lactate | Placebo         | No                                             | 0.0%                    | 0.0%                    |
| Johnell et al., 2002 (39) | Australia, Belgium, Canada, Italy, Mexico, South Africa, Spain, Sweden | 30            | 83/82                                         | 63.7/63.8    | 100% Alendronate | 10         | Placebo        | 10              | Yes                                             | 8.5%                    | 0.0%                    |
| Kung et al., 2000 (40)    | China          | 1             | 35/35                                         | 64.66       | 100% Alendronate | 10         | Placebo        | 12              | Yes                                             | 17.1%                   | 2.9%                    |
| Kushida et al., 2004 (41) | Japan          | 55            | 90/80                                         | 71.7/72.6    | 100% Alendronate | 5          | Alfacalcidol   | 36              | Yes                                             | 8.2%                    | 4.7%                    |
| Lanza et al., 2002 (42)   | United States of America | 5             | 126/126                                       | 54.7/54.7    | ND Alendronate  | 70         | Placebo        | 2.5             | Yes                                             | 22.2%                   | 0.4%                    |
| Lanza et al., 2000 (43)   | United States of America | 4             | 90/36                                         | 54.3/53.5    | 63.5% Alendronate | 40         | Placebo        | 1               | Yes                                             | ND                      | 7.1%                    |
| Lau et al., 2000 (44)     | China          | 1             | 35/47                                         | 74/74        | 100% Alendronate | 5          | Placebo        | 12              | Yes                                             | 12.0%                   | 0.0%                    |
| Leung et al., 2005 (45)   | China          | 4             | 31/34                                         | 67/67        | 100% Risedronate | 5          | Placebo        | 12              | Yes                                             | 3.1%                    | 0.0%                    |
| Liberman et al., 1995 (46) | USA, Canada, Australia, Europe, Israel, New Zealand, Mexico, South America | 28            | 175/355                                       | 64/64        | 100% Alendronate | 5          | Placebo        | 36              | Yes                                             | 0.0%                    | ND                      |
| McClung et al., 2001 (47) | North America, Europe, New Zealand, Australia | 183           | 3,093/3,134                                  | ND           | 100% Risedronate | 2.5        | Placebo        | 36              | No                                             | 17.0%                   | 2.1%                    |
| Miller et al., 2000 (48)  | United States of America | 38            | 88/84                                         | 67/67.1      | 100% Alendronate | 10         | Placebo        | 2               | No                                             | 14.0%                   | 2.3%                    |
| Murphy et al., 2001 (49)  | United States of America | 10            | 109/36                                       | 72.9/70.9    | 100% Alendronate | 10         | Placebo        | 18              | Yes                                             | 2.8%                    | 1.4%                    |
| Onvoll et al., 2000 (50)  | United States of America | 20            | 146/95                                        | 63/63        | 0% Alendronate   | 10         | Placebo        | 24              | Yes                                             | 15.8%                   | 0.8%                    |
| Pols et al., 1999 (51)    | Europe, Canada, Latin America, Australia, South Africa, China Europe, Australia | 153           | 950,958                                       | 62.8/62.8    | 100% Alendronate | 10         | Placebo        | 12              | Yes                                             | 20.8%                   | 3.7%                    |
| Reginster et al., 2000 (52) | Europe, Australia | 80            | 408/407                                      | 71/71        | 100% Risedronate | 2.5        | Placebo        | 24              | No                                             | 18.4%                   | 8.0%                    |
| Ryan et al., 2000 (53)    | United Kingdom | 2             | 41/41                                         | 65.6/66.1    | 90.1% Pamidronate | 150§       | Placebo        | 24              | Yes                                             | 54.3%                   | 0.0%                    |
| Seeman et al., 2010 (54)  | Argentina, Australia, Canada, France, USA | 9             | 81/83                                         | 60.7/60.8    | 100% Alendronate | 10         | Placebo        | 12              | No                                             | 54.9%                   | 0.0%                    |
| Shiraki et al., 1999 (55) | Japan          | 63            | 102/100                                      | 63.53/63.14  | 100% Alendronate | 5          | Alfacalcidol   | 12              | No                                             | 19.3%                   | 0.5%                    |
| Shiraki et al., 2003 (56) | Japan          | 70            | 52/54                                         | 60.7/60.5    | 99% Risedronate  | 1          | Placebo        | 3               | No                                             | 6.1%                    | 0.0%                    |
| Tucci et al., 1996 (57)   | United States of America | 18            | 98/192                                        | 66.5/64.2    | 100% Alendronate | 5          | Placebo        | 18              | Yes                                             | 14.0%                   | 1.4%                    |

(Continued)
Publication Bias
In case of non-severe side effects, both the visual assessment of the funnel plot and the Egger’s test, p=0.046, revealed small study effect, so the presence of publication bias was strongly suspected (Supplementary Figure 8). Therefore, the metanalytical pooled estimation was repeated by the use of trim and fill method, which did not change the overall risk association (RR= 0.99, CI: 0.91 ~ 1.07).

In case of severe side effects, publication bias was undetected by visual inspection of the funnel plot and Egger’s test p = 0.307 (Supplementary Figure 9).

Grade of Evidence
For non-severe GI side effects, the evidence was graded as very low due to inconsistency, indirectness, and publication bias and for severe GI side effects, the evidence was graded moderate due to indirectness (Table 2).

DISCUSSION
The pathophysiology of the bisphosphonate induced esophageal and gastric erosions has not been elucidated. In vitro studies suggest that the mucosal damage is produced through topical irritant effects on the gastric epithelium (60, 61). It is also described that BPs are competitively displacing the phospholipids from the mucus gel layer, therefore the mucosal hydrophobic barrier is attenuated and mucosal healing is hindered (62–64).

Our results from 42 RCTs with nearly 40,000 participants showed that the incidence of non-severe and severe GI side effects ranged between 0–54.9 and 0–10.3%, respectively. Neither the risk of non-severe nor the risk of severe adverse GI events was associated with the oral bisphosphonate use in osteoporotic patients.

Our meta-analysis is the first that objectified the risk of non-severe and severe GI side effects separately. When the use of bisphosphonates became widespread, it was predicted that gastroenterologists would see more patients with consequent GI problems (65).

Two previous reviews assessed the risk of GI side effects of risdonate and ibandronate separately (66, 67). A meta-analysis of nine RCTs focused on the GI tolerability of alendronate (68). A comprehensive network meta-analysis compared the GI safety of BPs, but they did not calculate the risk of side effects of bisphosphonates against placebo (69). The assessment of the risk of severe GI side effects was not based on the true severity of the GI side effects but how they were classified in the original studies.

Studies With a Primary Outcome of Gastrointestinal Adverse Events
Eight out of the included RCTs had GI adverse events as the primary outcome (18, 27, 43–42). Only two of these studies proved that the risk of non-severe GI side effects increased in patients taking BPs (18, 27). None of them showed an association between BPs and severe GI side effects.
**FIGURE 2** | Forest plot of non-severe adverse events.

| Study, year | RR (95% CI) | Events, Bisphosphonate | Events, Control | % Weight |
|-------------|-------------|-------------------------|----------------|----------|
| Liberman et al., 1995 | 0.41 (0.38, 0.60) | 39/957 | 63/957 | 2.69 |
| Greenough et al., 2002 | 0.71 (0.44, 1.13) | 26/224 | 37/226 | 1.89 |
| Felsenberg et al., 1997 | 0.81 (0.63, 1.08) | 57/219 | 72/225 | 3.78 |
| Downes et al., 2000 | 0.88 (0.46, 1.62) | 21/119 | 15/88 | 1.11 |
| Corder et al., 2005 | 0.87 (0.53, 1.43) | 26/224 | 30/226 | 1.74 |
| Kushida et al., 2004 | 0.89 (0.33, 2.42) | 79/10 | 780 | 0.47 |
| Shreiber et al., 1999 | 0.90 (0.51, 1.58) | 19/104 | 21/103 | 1.40 |
| McMurchie et al., 2001 | 0.96 (0.15, 6.05) | 10/416 | 529/3134 | 9.07 |
| Murphy et al., 2001 | 0.99 (0.83, 1.21) | 3/109 | 138 | 0.10 |
| Baur et al., 2000 | 0.99 (0.90, 1.09) | 1764/206 | 1750/223 | 10.41 |
| Kang et al., 2000 | 1.00 (0.92, 1.08) | 693 | 693 | 0.45 |
| Lenz et al., 2002 | 1.00 (0.93, 1.09) | 20/128 | 20/128 | 1.92 |
| Cummings et al., 1998 | 1.02 (0.91, 1.13) | 522/224 | 519/221 | 8.72 |
| Cherhal et al., 2004 | 1.02 (0.99, 1.06) | 412/296 | 251/295 | 7.28 |
| Black et al., 1996 | 1.03 (0.93, 1.14) | 443/322 | 424/359 | 8.89 |
| Flaggman et al., 2000 | 1.03 (0.74, 1.44) | 83/361 | 40/160 | 3.19 |
| Regnier et al., 2000 | 1.05 (0.62, 1.34) | 160/818 | 76/647 | 4.69 |
| Poli et al., 1999 | 1.08 (0.81, 1.42) | 209/890 | 191/898 | 6.30 |
| Yan et al., 2006 | 1.09 (0.75, 1.60) | 47/280 | 43/280 | 2.63 |
| Leung et al., 2005 | 1.10 (0.77, 1.60) | 131 | 134 | 0.07 |
| Mambri et al., 1999 | 1.10 (0.93, 1.31) | 206/813 | 189/815 | 6.60 |
| Miller et al., 2000 | 1.13 (0.54, 2.38) | 138/88 | 115/84 | 0.83 |
| Boonen et al., 2000 | 1.19 (0.77, 2.84) | 23/104 | 9/5 | 0.85 |
| Lau et al., 2000 | 1.24 (0.42, 3.85) | 753 | 547 | 0.41 |
| Ritt et al., 2006 | 1.25 (0.52, 2.98) | 15/92 | 9/41 | 0.62 |
| Ascari-Evans et al., 2003 | 1.29 (0.35, 3.11) | 15/95 | 6/49 | 0.61 |
| Seeman et al., 2010 | 1.34 (1.10, 1.78) | 51/181 | 50/183 | 3.96 |
| Bell et al., 2002 | 1.40 (0.70, 2.61) | 13/33 | 9/32 | 0.94 |
| Borne et al., 2000 | 1.48 (0.87, 2.57) | 19/92 | 73/92 | 0.74 |
| Esam et al., 2004 | 1.48 (0.29, 2.63) | 27/125 | 16/224 | 1.36 |
| Tucci et al., 1996 | 1.53 (1.01, 2.62) | 51/286 | 21/192 | 1.84 |
| Hogling et al., 2005 | 1.73 (1.39, 2.17) | 34/44 | 0/108 | 0.06 |
| Joffre et al., 2002 | 1.78 (0.62, 5.08) | 9/3 | 5/3 | 0.43 |
| Orr et al., 2003 | 1.82 (0.59, 5.58) | 28/146 | 10/36 | 1.03 |
| Criner et al., 2001 | 2.16 (1.44, 3.23) | 61/224 | 20/230 | 2.40 |
| Shreiber et al., 2005 | 4.15 (1.01, 16.59) | 24/154 | 254 | 0.25 |
| Clementsen et al., 1997 | 4.55 (0.35, 62.67) | 439 | 0/44 | 0.05 |
| Aedch et al., 2009 | 24.54 (1.52, 400.55) | 242/291 | 0/147 | 0.02 |
| Overall (unadjusted) | 1.05 (0.99, 1.12) | 4962/2375 | 4465/1694 | 100.00 |

**NOTE:** Weights are from random effects analysis.

**FIGURE 3** | Forest plot of severe adverse events.
While the first trial of Cryer et al. managed to detect an increased risk of non-severe GI side effects, their second trial, in which approximately half of participants took non-steroidal anti-inflammatory drugs on both arms, could not demonstrate this association (27, 28).

A study in 2,000 assessed each participants’ GI side effects through endoscopic inspection of the mucosa at baseline and completion of the study. They concluded that mucosal damage did not translate into clinically significant symptoms or side effects (43).

Miller et al. investigated whether previous GI side effects of BP therapy predisposed to recurrent side effects after rechallenge with alendronate. They found no significant risk of severe or non-severe GI side effects associated with the alendronate use. The incidence of non-severe and severe GI side effects were 14 and 2.3%, respectively (48).

Since the introduction of the BPs in the treatment of osteoporosis multiple studies confirmed their GI tolerability. Even if they have non-severe GI side effects, their use rarely results in severe complications needing the attention of the gastroenterologist.

Oral BPs are nowadays recommended to take with water and to avoid lying down after intake to avoid esophageal irritation. These precautions might reduce the incidence of upper GI side-effects in more recent RCTs and in current clinical practice.

**Strength of Our Study**

Our work, which includes a large number of RCT-s and participants was conducted following a rigorous methodology. Furthermore, most of the included RCT-s are multinational and multicentric. To date, this is the first meta-analysis which quantified the risk of non-severe and severe GI side effects of oral BP therapy.

**Limitations of Our Study**

In most of the studies, GI side effects were a secondary outcome and were not powered statistically to reveal a significant difference in that respect. The heterogeneity of the strategy of vigilance for side effects probably explains the wide range of incidence of side effects; however, it did not translate to statistical heterogeneity among the severe side effects. The differences between sexes, ages, length of the studies and various definitions in addition to different approaches of the screening for non-severe side effects resulted in moderate and significant heterogeneity among the studies. Also, the included studies likely used different sets of predetermined GI side effects during the screening for side effects. Another significant limitation of the study is that in 24 of 42 RCTs included in the analysis, pre-existing and/or previous GI diseases were exclusion criteria. The conclusions drawn from the meta-analysis are therefore restricted to selected populations, and the results must be interpreted with caution. These considerations are reflected in Table 2, in which the grades of evidence were rated very low for non-severe GI side-effects and only moderate for severe GI side-effects.

Risk assessment revealed unclear bias in most of the studies concerning the reporting of the results.

**FIGURE 4 | (A, B) Subgroup analysis of trials with the primary outcome of GI tolerability of BP therapy.**
CONCLUSION

Implications for Research

Although the results suggest that bisphosphonates do not increase the risk of GI side effects in the general osteoporotic population, we cannot conclude whether they are safe to use in a high-risk population with preexisting GI pathologies (e.g., gastroesophageal reflux, peptic ulcer disease, etc.). Therefore future phase III trials should focus on these high-risk populations.

Implications for Practice

Bisphosphonates seem to be safe in the osteoporotic population concerning the GI side effects, however other factors need to be considered when decisions on treatment are made.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

ZD, BE, and PH conceived the study. ZD, NV, and SK wrote the protocol. ZD, EC, and LS did the literature search. ZD and NV screened the records and extracted data. LS and AP validated the extracted data. ZD, NV, and EC assessed the quality of the included studies. LH did the statistical analysis. ZD and NV prepared the tables. BE, ZD, and NV wrote the first draft of this manuscript. PH, SK, and AP supervised the manuscript and approved the submitted draft. BE is the guarantor of this paper. All authors contributed to the article and approved the submitted version.

FUNDING

Sponsors were not involved in the design, data collection, analysis, interpretation, or preparation of the manuscript. Financial support: Supported by the Economic Development and Innovation Operative Programme Grant (GINOP-2.3.2-15-2016-00048) and the Human Resources Development Operational Programme Grants (EFOP-3.6.2-16-2017-00006).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.573976/full#supplementary-material

SUPPLEMENTARY FIGURE 1 | Non-severe adverse events subgroup by active substance.

SUPPLEMENTARY FIGURE 2 | Severe adverse events subgroup by active substance.

SUPPLEMENTARY FIGURE 3 | Non-severe GI side effects subgroup by more than 24 months of treatment.

SUPPLEMENTARY FIGURE 4 | Severe GI side effects subgroup by more than 24 months of treatment.

TABLE 2 | Grade of evidence.

| Outcomes                              | Anticipated absolute effects* (95% CI) | Relative effect (95% CI) | % of participants (studies) | Certainty of the evidence (GRADE) | Importance |
|---------------------------------------|---------------------------------------|--------------------------|-----------------------------|----------------------------------|-----------|
|                                       | Risk with Control | Risk with Bisphosphonate | RR                          |                                |           |
| Non-severe GI adverse events          | 271 per 1,000          | 285 per 1,000            | (266 to 304)                | 38,769                           | VERY LOW |
|                                       |                        |                         | RR 1.05                     |                                | IMPORTANT |
| Severe GI adverse events              | 40 per 1,000           | 41 per 1,000             | (37 to 45)                  | 37199                            | MODERATE  |
|                                       |                        |                         | RR 1.01                     |                                | CRITICAL  |

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

CI, confidence interval; RR, risk ratio.

GRADE Working Group grades of evidence

High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.

Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of the effect.

Heterogeneity was moderate among the studies included in the analysis of the risk of non-severe GI adverse events (I² = 48.1%, p = 0.001).

There were major differences in the intervention groups of the studies included regarding the used drug (alendronate/ibandronate/risedronate/pamidronate), dosage, and administration intervals.

The funnel plot of this outcome revealed asymmetry and Egger’s test suggested small study effect (p = 0.046).
commonly used BP therapies: 70 mg/week and 10 mg/day alendronate per os.

REFERENCES

1. Nuti R, Brandi ML, Checchia G, Di Munno O, Dominguez L, Falaschi P, et al. Guidelines for the management of osteoporosis and fragility fractures. Intern Emerg Med (2019) 14(1):85–102. doi: 10.1007/s11739-018-1874-2

2. Kanis JA, Cooper C, Rizzoli R, Reginster JY. Scientific Advisory Board of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) and the Committee of Scientific Advisors of the International Osteoporosis Foundation (IOF), et al. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int (2019) 30(1):3–44. doi: 10.1007/s00198-018-4704-5

3. Kennel KA, Drake MT. Adverse Effects of Bisphosphonates: Implications for Osteoporosis Management. Mayo Clin Proc (2009) 84(7):632–8. doi: 10.4065/84.7.632

4. Payer J, Killinger Z, Ivana S, Celec P. Therapeutic adherence to bisphosphonates. Biomed Pharmacother = Biomed Pharmacother (2007) 61(4):191–3. doi: 10.1016/j.biopha.2007.02.003

5. Goldshtein I, Rouach V, Shamir-Stein N, Yu J, Chodick G. Role of Side Effects, Physician Involvement, and Patient Perception in Non-Adherence with Oral Bisphosphonates. Adv Ther (2016) 33(8):1374–84. doi: 10.1007/s12325-016-0360-3

6. Rossini M, Adami G, Adami S, Viapiana O, Gatti D. Safety issues and adverse reactions with osteoporosis management. Expert Opin Drug safety (2016) 15(3):321–32. doi: 10.1007/s13492-015-0326-8

7. de Groen PC, Lubbe DF, Hirsch LJ, Daifotis A, Stephenson W, Freedholm D, et al. Esophagitis Associated with the Use of Alendronate. N Engl J Med (1996) 335(14):1016–21. doi: 10.1056/NEJM199603313551403

8. Ribeiro A, Devault KR, Wolfe J, Stark ME. Alendronate-associated esophagitis: endoscopic and pathologic features. Gastrointestinal Endoscopy (1998) 47(6):525–8. doi: 10.1016/S0016-5107(98)70256-1

9. Nagano Y, Matsui H, Shimokawa O, Hirayama A, Nakamura Y, Tamura M, et al. Bisphosphonate-induced gastrointestinal mucosal injury is mediated by mitochondrial superoxide production and lipid peroxidation. J Clin Biochem Nutr (2012) 51(3):196–203. doi: 10.3164/jcbn.12-41

10. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev (2016) 5(1):1. doi: 10.1186/s40604-016-0453-4

11. https://www.fda.gov/safety/reporting-serious-problems-fda/what-serious-adverse-event?fbclid=IwAR2tfSlOW5y4ZsbUjT4D_ky7MV_
...
31. Eisman JA, Rizzoli R, Roman-Ivorra J, Lipschitz S, Verbruggen N, Gaines KA, et al. Upper gastrointestinal and overall tolerability of alendronate once weekly in patients with osteoporosis: Results of a randomized, double-blind, placebo-controlled study. *Curr Med Res Opin* (2004) 20(5):699–705. doi: 10.1185/03007990425003548

32. Felsenberg D, Alenfeld F, Bock O, Hammermeister C, Gowan Wh Te Fosti-Study Group. Placebo-controlled multicenter study of oral alendronate in postmenopausal osteoporotic women. *Maturitas* (1998) 31(1):35–44. doi: 10.1016/S0378-5122(98)00050-4

33. Fogelman I, Ribot C, Smith R, Ethgen D, Sod E, Reginster JY. Risedronate vs. oral alendronate on bone mineral density and the incidence of fractures in women with postmenopausal osteoporosis. *Maturitas* (2005) 36(2):358–10. doi: 10.1016/j.maturitas.2000.08.009

34. Greenspan S, Field-Munves E, Tonino R, Smith M, Petruschke R, Wang L, et al. Efficacy and tolerability of alendronate and risedronate: A randomized, placebo-controlled study. *Mayo Clin Proc* (2002) 77 (10):1044–52. doi: 10.4065/77.10.1044

35. Harris ST, Watts NB, Genant HK, Genant HK, Hangartner T, Keller M, et al. Effects of raloxifene and alendronate on bone density and biochemical markers of bone turnover in postmenopausal osteoporotic women. *N Engl J Med* (2001) 345(6):487–92. doi: 10.1056/nejm200102013450503

36. Liberman UA, Weiss SR, Bröll J, Minne HW, Quan H, Bell NH, et al. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. *N Engl J Med* (1995) 333(22):1437–43. doi: 10.1056/NEJM19951133332201

37. McClung MR, Geusens P, Miller PD, Zippel H, Rensen WG, Roux C, et al. Effect of risedronate on the risk of hip fracture in elderly women. Hip Intervention Program Study Group. *N Engl J Med* (2001) 344(5):333–40. doi: 10.1056/nejm200102133440503

38. Miller PD, Woodson G, Licata AA, Ettinger MP, Mako B, Smith ME, et al. Rechallenge of patients who had discontinued alendronate therapy because of upper gastrointestinal symptoms. *Clin Ther* (2002) 22(12):1433–42. doi: 10.1016/S0149-2918(00)80342-8

39. Murphy MG, Weiss S, McClung M, Schnitzer T, Cerchiosi K, Conner J, et al. Effect of alendronate and MK-677 (a growth hormone secretagogue), individually and in combination, on markers of bone turnover and bone mineral density in postmenopausal osteoporotic women. *J Clin Endocrinol Metab* (2001) 86(3):1116–23. doi: 10.1210/jcem.86.3.7294

40. Orwoll E, Ettinger M, Weiss S, Miller P, Kendler D, Graham J, et al. Alendronate for the treatment of osteoporosis in men. *N Engl J Med* (2000) 343(9):604–10. doi: 10.1056/NEJM200003134300902

41. Pola HAP, Felsenberg D, Hanley DA, Stepan J, Muñoz-Torres M, Wilkin TJ, et al. Multinational, placebo-controlled, randomized trial of the effects of alendronate on bone density and fracture risk in postmenopausal women with low bone mass: Results of the FOSIT study. *Osteoporos Int* (1999) 9(5):461–8. doi: 10.1007/PL00004179

42. Register JY, Minne HW, Sorensen OH, Hooper M, Roux C, Brandi ML, et al. Randomized trial of the effects of risedronate on vertebral fractures in women with established postmenopausal osteoporosis. *Osteoporos Int* (2000) 11 (1):83–91. doi: 10.1007/s001980050050

43. Ryan M, Blake GM, Davie M, Haddaway M, Gibson T, Fogelman I. Intermittent oral disodium pamidronate in established osteoporosis: A 2 year double-masked placebo-controlled study of efficacy and safety. *Osteoporos Int* (2000) 11(2):171–6. doi: 10.1007/PL00004179

44. Seeman E, Delmas PD, Hanley DA, Sellmeyer D, Cheung AM, Shane E, et al. Microarchitectural deterioration of cortical and trabecular bone: differing effects of denosumab and alendronate. *J Bone Min Res* (2010) 25(8):1886–94. doi: 10.1002/jbmr.81

45. Shiraki M, Kushida K, Fukunaga M, Kishimoto H, Taga M, Nakamura T, et al. A double-masked multicenter comparative study between alendronate and alfalcacidol in Japanese patients with osteoporosis. *Osteoporos Int* (1999) 10 (3):183–92. doi: 10.1007/s001980050214

46. Shiraki M, Fukunaga M, Kushida K, Higashitani Y, Haddaway M, Gibson T, Fogelman I. Alendronate prevents bone loss in Chinese post-menopausal women at risk of osteoporosis. *Climacteric* (2002) 5(4):221–9. doi: 10.1080/136971002101246568

47. Shiraki M, Kushida K, Higashitani Y, Haddaway M, Gibson T, Fogelman I. Alendronate reduces vertebral fracture risk in postmenopausal Japanese women with osteoporosis: A 3-year follow-up study. *J Bone Miner Metab* (2002) 22(5):462–8. doi: 10.1007/s00774-004-0508-0

48. Lanza F, Sahab B, Schwartz H, Winograd S, Torosis J, Quan H, et al. The upper GI safety and tolerability of oral alendronate at a dose of 70 milligrams once weekly: A placebo-controlled endoscopy study. *Am J Gastroenterol* (2002) 97 (1):58–64. doi: 10.1111/j.1572-0241.2002.00546.x

49. Lanza F, Schwartz H, Sahab B, Malaty HM, Musliner T, Reyes R, et al. An endoscopic comparison of the effects of risedronate and alendronate on upper gastrointestinal mucosa. *Am J Gastroenterol* (2000) 95(11):3121–7. doi: 10.1111/j.1572-0241.2000.00531-7

50. Lau EM, Woo J, Chan YH, Griffith J. Alendronate prevents bone loss in Chinese women with osteoporosis. *Bone* (2000) 27(5):677–80. doi: 10.1016/s8756-3282(00)00378-1

51. Leung JYY, Ho AYY, Ip TP, Lee G, Kung AWC. The efficacy and tolerability of risedronate on bone mineral density and bone turnover markers in osteoporotic Chinese women: A randomized placebo-controlled study. *Bone* (2005) 36(2):358–64. doi: 10.1016/j.bone.2004.10.014

52. Liberman UA, Weiss SR, Broll J, Minne HW, Quan H, Bell NH, et al. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. *J Bone Miner Res* (1999) 13(5):461–8. doi: 10.1007/s001980050133

53. McClung MR, Geusens P, Miller PD, Zippel H, Rensen WG, Roux C, et al. Effect of risedronate on the risk of hip fracture in elderly women. Hip Intervention Program Study Group. *J Bone Min Res* (2001) 16(10):1792–7. doi: 10.1002/jbmr.81

54. Elliott SN, McKnight W, Davies NM, MacNaughton WK, Wallace J. Alendronate induces gastric injury and delays ulcer healing in rodents. *Life Sci* (1997) 62(1):77–81. doi: 10.1016/s0022-3205(97)00140-0

55. Thomson ABR, Appleman S, Keelan M, Wallace JL. Role of Gastric Mucosal Concentrations in Development of Bisphosphonate Damage to Gastric Mucosa. *Digestive Dis Sci* (2003) 48 (2):308–14. doi: 10.1023/A:1021979510860

56. Lichtenberger LM, Romero JJ, Gibson GW, Blank MA. Effect of bisphosphonates on surface hydrophobicity and phosphatidylcholine concentration of rodent gastric mucosa. *Digestive Dis Sci* (2000) 45 (9):792–801. doi: 10.1023/A:1005574009856

57. Pazianas M, Abrahamsen B. Safety of bisphosphonates. *Bone* (2011) 49 (1):103–10. doi: 10.1016/j.bone.2011.01.02

Frontiers in Endocrinology | www.frontiersin.org
November 2020 | Volume 11 | Article 573976
65. Graham DY. What the gastroenterologist should know about the gastrointestinal safety profiles of bisphosphonates. *Digestive Dis Sci* (2002) 47(8):1665–78. doi: 10.1023/a:1016495221567

66. Taggart H, Bolognese MA, Lindsay R, Ettinger MP, Mulder H, Josse RG, et al. Upper gastrointestinal tract safety of risedronate: a pooled analysis of 9 clinical trials. *Mayo Clin Proc* (2002) 77(3):262–70. doi: 10.4065/77.3.262

67. Epstein S, Delmas PD, Emkey R, Wilson KM, Hiltbrunner V, Schimmer RC. Oral ibandronate in the management of postmenopausal osteoporosis: Review of upper gastrointestinal safety. *Maturitas* (2006) 54(1):1–10, doi: 10.1016/j.maturitas.2006.01.011

68. Zhou M, Zheng Y, Li J, Wu J, Xu W, Cui L, et al. Upper gastrointestinal safety and tolerability of oral alendronate: A meta-analysis. *Exp Ther Med* (2016) 11 (1):289–96. doi: 10.3892/etm.2015.2848

69. Tadrous M, Wong L, Mamdani MM, Juurlink DN, Krahn MD, Lévesque LE, et al. Comparative gastrointestinal safety of bisphosphonates in primary osteoporosis: a network meta-analysis. *Osteoporos Int* (2014) 25(4):1225–35. doi: 10.1007/s00198-013-2576-2

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Dömötör, Vörhendi, Hanaá, Hegyi, Kiss, Csáki, Szakó, Párniczky and Éróss. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.