

Are Granulocyte Colony-Stimulating Factors Beneficial in Treating Diabetic Foot Infections?

A meta-analysis

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OBJECTIVE — To assess the value of granulocyte colony-stimulating factor (G-CSF) as adjunctive therapy for diabetic foot infections.

RESEARCH DESIGN AND METHODS — We systematically searched the medical literature (including Medline, Embase, LookSmart, and the Cochrane Library) for prospective randomized studies that used G-CSF as an adjunct to standard treatment for diabetic foot infections. Using a conventional meta-analysis, we pooled the relative risks (RRs) for outcomes of interest, including resolution of infection, wound healing, duration of antibiotic therapy, and need for various surgical interventions, using a fixed-effects model.

RESULTS — Five randomized trials, with a total of 167 patients, met our inclusion criteria. The methodological quality of the studies was satisfactory. The investigators administered various G-CSF preparations parenterally for between 3 and 21 days. The meta-analysis revealed that adding G-CSF did not significantly affect the resolution of infection or the healing of the wounds but was associated with a significantly reduced likelihood of lower extremity surgical interventions (RR 0.38 [95% CI 0.20–0.69], number of patients who needed to be treated: 4.5), including amputation (0.41 [0.17–0.95], number of patients who needed to be treated: 8.6). There was no evidence of heterogeneity among the studies or of publication bias, suggesting that these conclusions are reasonably generalizable and robust.

CONCLUSIONS — Adjunctive G-CSF treatment does not appear to hasten the clinical resolution of diabetic foot infection or ulceration but is associated with a reduced rate of amputation and other surgical procedures. The small number of patients who needed to be treated to gain these benefits suggests that using G-CSF should be considered, especially in patients with limb-threatening infections.

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Foot infections in patients with diabetes can be difficult to treat, and therapeutic failure often leads to a lower-extremity amputation (1,2). These infections may be refractory to treatment

for several reasons, including inadequate surgical interventions, suboptimal wound care, or severe limb ischemia (3). All infected foot lesions require antibiotic therapy, but their penetration to infected

soft tissue and bone may be inadequate, and the incidence of antibiotic resistance is increasing (4). Furthermore, diabetes may cause immunological deficiencies, including abnormal neutrophil chemotaxis, phagocytosis, and intracellular killing (5–7). These factors help explain reported clinical failure rates for diabetic foot infections of 20–30% (3,5–7). Thus, several investigators have sought adjunctive therapies for treating these potentially severe infections.

Granulocyte colony-stimulating factor (G-CSF) is an endogenous hematopoietic growth factor that induces terminal differentiation and release of neutrophils from the bone marrow (8). G-CSF stimulates the growth and improves the function of both normal and defective neutrophils (9), including in patients with diabetes (10). It appears to play a central role in the normal host response to infection (11), including having immunomodulatory and antibiotic-enhancing functions (12). In its purified, cloned recombinant form, commercially approved G-CSF has been used to treat various difficult infectious problems (13–15). In nonneutropenic patients, G-CSF may stimulate neutrophil production, enhancing the inflammatory response (16,17).

Because G-CSF specifically enhances neutrophil functions in diabetic patients (10), including those with foot infections (18), several investigators have explored using it as an adjunct to treating diabetic foot infections. Unfortunately, there have only been a few studies and each enrolled a relatively small numbers of subjects. Furthermore, the available studies have come to different conclusions regarding the usefulness of G-CSF. In such situations, meta-analysis is a useful tool to determine the potential benefit of a therapeutic intervention (19–22). Thus, to define the effectiveness of G-CSF as an adjunctive therapy for treating diabetic foot infections, we thoroughly searched the literature for all prospective studies of

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this issue then subjected these to a systematic review and meta-analysis.

RESEARCH DESIGN AND METHODS

We searched the medical literature, using Medline, Embase, LookSmart's Find Articles, and the Cochrane Library, for relevant studies published between January 1990 and July 2003. MeSH terms used were "granulocyte colony-stimulating factor (or G-CSF)" and "diabetic foot." We supplemented the computer search by reviewing as many diabetic foot online websites and published bibliographies as we could find, hand searching the bibliographies from the articles retrieved, reviewing relevant meeting abstracts, and asking study authors and other experts in the field about any additional published or unpublished studies on this topic.

Study selection, quality assessment, and data extraction

We included in our analysis only prospective randomized studies whose main purpose was to investigate the therapeutic effects of G-CSF in diabetic foot infections. Studies were included only if they compared the efficacy of standard treatment alone with that of standard treatment plus adjunctive G-CSF therapy. We assessed the quality of each trial with a scale developed by Jadad et al. (23) that scores (from a low of zero to a high of five) the randomization, double blinding, and reports of dropouts and withdrawals.

Data extracted from each study included the following: clinical outcomes related to both curing the infection (resolution of cellulitis or other signs and symptoms of infection) and healing of any foot ulcer, the duration of antibiotic therapy (by any route) provided, the duration of hospitalization, the need for any type of lower-extremity amputation or other major surgical procedures, and the need for vascular (arterial) surgery or angioplasty. We also sought information on the changes in blood leukocyte count and any side effects of G-CSF treatment. Two reviewers (M.C. and F.D.L.) independently examined the data and resolved disagreements of interpretation by discussion.

When a publication had missing or incomplete information, we attempted to contact the author(s). In three instances, they provided additional data that we added to our tables. Thus, in some instances, the results presented in our tables

differs from those shown in the published articles.

Statistical analysis

We conducted a conventional meta-analysis using the Mantel-Haenszel fixed-effects model (24), applying the Der Simonian and Laird random-effects model only in cases where the homogeneity hypothesis was rejected (25,26). We calculated both the study-specific and common 95% CIs by the method of Woolf (27) and used risk ratio (RR) as a measure of the effect size. To calculate the number of patients who needed to be treated to prevent one event, we assessed the pooled risk differences (28).

For continuous variables (e.g., neutrophil count and duration of antibiotic treatment), we used the weighted mean difference. The weight assigned to each study (i.e., how much influence it had on the overall meta-analysis results) was determined by the precision of its estimate of effect, which is equal to the inverse of the variance. This method assumes that all of the trials have measured the outcome on the same scale.

Assessment of publication bias and heterogeneity

To visually inspect for publication bias, we generated graphical funnel plots (29). The statistical methods used to detect funnel-plot asymmetry were the rank correlation test of Begg and Mazumdar (30) and the regression asymmetry test of Egger et al. (29). Because the relative merits of the two available methods are not well established, we used both.

We assessed the heterogeneity of results of the studies by using the Cochran's Q test (31,32). This test, however, has a low power for detecting heterogeneity when the number of studies included in the meta-analysis is small. Thus, we also used the recently introduced quantity, I^2 (33), which is calculated from the usual test statistics and provides a less-biased measure of the degree of inconsistency across studies in a meta-analysis. There was neither external funding nor any sponsorship for this study.

RESULTS

Description of studies and methodological quality

Our literature search uncovered 17 articles (8,18,34–48) with information

about using G-CSF for diabetic foot problems. These included one systematic review, seven traditional reviews, seven clinical studies, a comment letter on one of these studies, and one case report. The systematic review of treating foot ulcers in diabetic patients was published in 1999 (34) and only included one study (published in 1997) using subcutaneous G-CSF. One placebo-controlled trial (48) with granulocyte-macrophage colony-stimulating factor examined its effect on healing uninfected ulcers. Thus, there were five prospective randomized studies (8,35–38) using G-CSF for infected diabetic foot lesions that met the predefined inclusion criteria.

Table 1 summarizes the main elements of the protocol, patient characteristics, and major outcomes of the five included studies. In each study, the enrolled patients were hospitalized for treatment. The details provided by the authors on the clinical characteristics of the infections varied, but the described severity among the studies ranged from relatively mild (36,38) to severe (35). Patients with sepsis, gangrene, or deep soft tissue infection were generally not enrolled. Initial antibiotic therapy was apparently mostly parenteral and in most studies not modified by culture results. The specific regimens and duration of therapy varied, but in four studies, it consisted of a fluoroquinolone (ciprofloxacin or ofloxacin) combined with an antianaerobic drug (clindamycin or metronidazole). The inclusion and exclusion criteria also varied, but all required that the infections were severe enough to warrant hospitalization, and they were usually classified as Wagner grade 2 or 3 (49). Patients receiving immunosuppressive therapy or with immunosuppressive disorders, critical limb ischemia, hepatic or renal insufficiency, or hematological disorders were excluded in each study.

The G-CSF preparation used was filgrastim in four studies and lenograstim in one. It was administered subcutaneously in four studies and intravenously in one. In each study, the G-CSF preparation was held, or its dose reduced, if the neutrophil count increased beyond a previously set value. The duration of G-CSF therapy varied from 3 to 21 days.

The Jadad scores for quality of the studies ranged from 1 to 5; the mean was 3.4, and four trials had a score ≥ 3 . Four studies (8,35,37,38) reported conceal-

Table 1—Main characteristics of randomized studies that compared the efficacy of treating diabetic foot infections with standard treatment alone (control subjects) versus with added G-CSF therapy

Reference [author (ref. no.), country]	Clinical presentation	Type of G-CSF therapy	Study design (randomized, plus)	Patients enrolled (n)	Main outcomes*	Comments
Gough (8), U.K.†‡	Extensive cellulitis	Filgrastim 5 µg/kg s.c. daily for 7 days	Double blind, placebo controlled	Screened 57, G 20, C 20	Inf: G 7, C 12, P = 0.02; Surg: G 0, C 4, P = NS; Hosp: G 10, C 18, P = 0.02	Other significantly improved outcomes with G-CSF§
de Lalla (35), Italy	Severe limb-threatening infection	Lenograstim 263 µg s.c. daily for 21 days	Evaluator blind, not placebo controlled	G 20, C 20	Inf: G 12, C 9, P = NS; Surg: G 3, C 9, P = 0.04	All had osteomyelitis. Similar rates of pathogen eradication
Yönem (36), Turkey	Pedal cellulitis or Wagner grade ≤2 lesion	Filgrastim 5 µg/kg s.c. daily for ≥3 days	Not blinded or placebo controlled	G 15, C 15	Inf: G 24, C 22, P = NS; Surg: G 2, C 3, P = NS; Hosp: G 27, C 28, P = NS	Duration of intravenous antibiotic NS
Kästenbauer (37), Austria†‡	Infected foot ulcer, Wagner grades 2–3	Filgrastim 5 µg/kg s.c. daily for 10 days	Patient blind, placebo controlled	Screened 73, G 20 (18), C 17 (16)¶	Inf: G 77%, C 66%, P = NS ; Surg: G 1, C 1, P = NS	Number of vascular procedures and duration of intravenous antibiotics were similar.
Viswanathan (38), India†	Extensive cellulitis, Wagner grades 2–3	Filgrastim 5 µg/kg i.v. daily for 7 days	Double blind, placebo controlled	G 10, C 10	Inf: G 9, C 3, P = NS#; Surg: G 0, C 3, P = NS; Hosp: G 7.4, C 8.8, P = 0.02	Most patients had peripheral vascular disease.

*Number of patients and number of hospital days. †Sponsored by Amgen. ‡Author provided additional unpublished details. §Withdrawal of intravenous antibiotics, time to negative swab culture, diminution of foot temperature, and number of vascular procedures. ¶Number who completed the study in parentheses. ||Percent reduction in infection summary score. #Number with improved cellulitis on day 7 of therapy. C, control patients; G, G-CSF-treated patients; Hosp, number of days hospitalized; Inf, number in whom infection resolved; NS, not statistically significant; Surg, number who required amputation or other surgical intervention.

ment of treatment allocation, but only three gave placebo injections, and only two described employing a double-blind design.

Main results

A total of 167 patients were included in the five randomized studies, 85 in the G-CSF-treated group and 82 in the control group. There was no evidence of an imbalance in baseline patient demographic or clinical characteristics in any study. Because the trials did not uniformly report the results of some clinical outcomes of interest, we can only present them descriptively.

The meta-analysis showed that adding G-CSF injections to standard treatment did not significantly affect the clinical resolution of infection or the likelihood or rate of healing of wounds. It did, however, statistically significantly reduce the likelihood of undergoing a surgical procedure. Figure 1 shows the RRs and 95% CIs for individual studies for the outcomes of “amputation” and for overall invasive interventions, which included amputation as well as extensive debridement, angioplasty, and other vascular surgical procedures. Table 2 shows the cumulative RR and related 95% CI, pooled rates, percent risk reduction, and the number of patients who needed to be treated to prevent these adverse outcomes. The RRs in favor of the G-CSF group were 0.41 (95% CI 0.17–0.95; $z = 2.08$, $P = 0.038$) for amputation and 0.38 (0.20–0.69; $z = 3.13$, $P = 0.002$) for overall invasive interventions.

The overall duration (in days) of intravenous antibiotic therapy varied widely, with a mean of ~16 days in each group. Using the fixed-effects model, the weighted mean difference favoring the G-CSF group of -0.36 days (95% CI -1.39 to 0.67 , $P = 0.49$) was not statistically significant. As would be expected, the leukocyte count during therapy was higher in the G-CSF group ($30.81 \times 10^9/l$ [95% CI 14.87–46.75]) than in control group ($5.03 \times 10^9/l$ [4.12–5.94]). There was evidence of heterogeneity among studies regarding this outcome, but this is primarily accounted for by the variations in dosages and duration of G-CSF and the different times during treatment at which the investigators assessed the leukocyte count. Using the random-effects model, the weighted mean difference in leukocyte count between the groups was

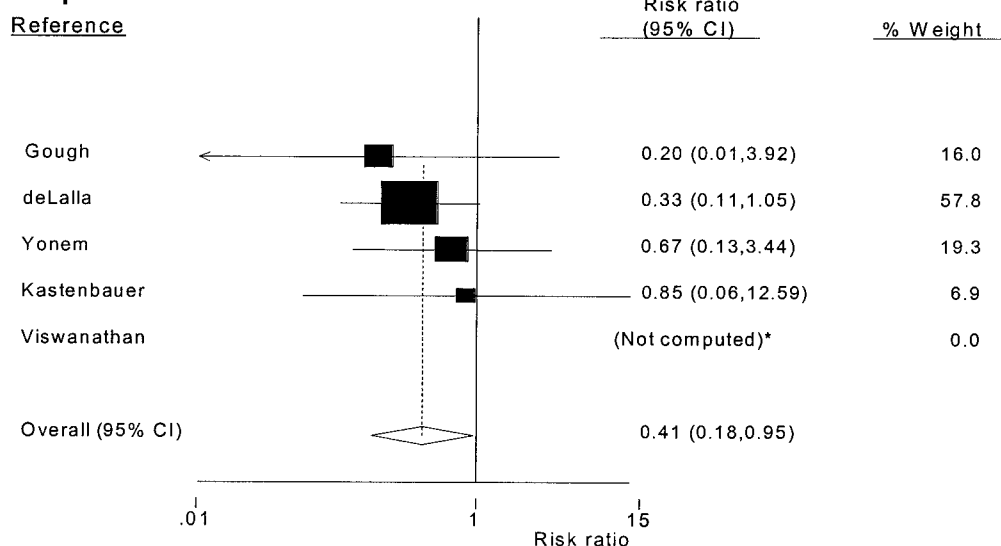
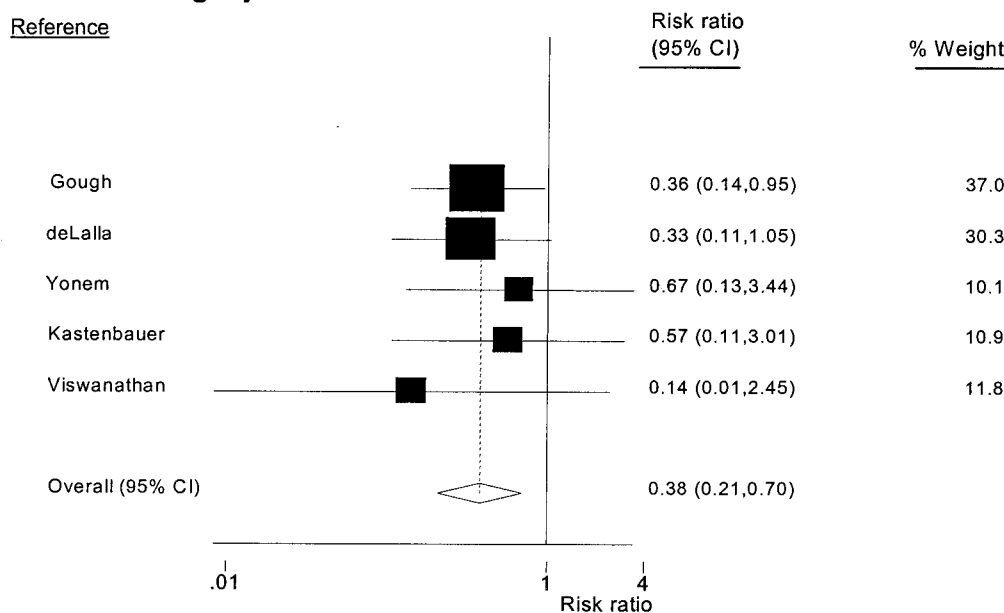
A Amputation**B Overall Surgery**

Figure 1— Pooled RR estimates and their 95% CIs for the outcomes “amputation” (A) and “overall surgery” (B). Studies are identified by name of the first author. Size of squares is proportional to Mantel-Haenszel weighted risk ratio. *Cannot be computed because the presence of frequencies equals zero.

$25.24 \times 10^9/l$ (9.57–40.92, $P = 0.002$). None of the studies reported any significant adverse effects of G-CSF therapy.

Heterogeneity and publication bias assessment

With the exception of the neutrophil count data, there was no evidence of in-

tertrial heterogeneity for the outcomes analyzed. Values of I^2 (with their 95% uncertainty intervals) were 0% (0–53%) for amputation and 0% (0–30%) for overall surgical interventions, indicating no observed heterogeneity. There was also no evidence for publication bias, as shown in Fig. 2, by the symmetrical appearance of

the Begg’s funnel plots for both outcomes of interest. The Begg-Mazumdar and Egger tests also showed no evidence of publication bias (Begg’s test: adjusted Kendall’s score = 0, SD of score = 2.94, $z = 0$, $p[z] = 1$, continuity corrected $z = -0.34$, $p[z] = 1$; Egger’s test: $t = 0.30$, $p[t] = 0.790$).

Table 2—Need for lower-extremity amputation and for overall invasive interventions (including amputations) for patients with diabetic foot infections treated with standard treatment plus G-CSF versus standard treatment alone (control subjects)

Treatment group	Crude rate [n/total in group (%)]	Pooled rate	RR (95% CI)	Risk reduction [% (95% CI)]*	NNT (95% CI)
Lower extremity amputations					
G-CSF subjects	6/85 (7.0)	8.2	0.41 (0.17–0.95)	11.6 (1.9–15.7)	8.6 (6.4–52.5)
Control subjects	15/82 (18.2)	13.3			
Overall invasive interventions					
G-CSF subjects	11/85 (12.9)	13.6	0.38 (0.20–0.69)	22.3 (11.1–28.8)	4.5 (3.5–9.0)
Control subjects	29/82 (35.3)	32.0			

*Calculated based on the crude rate in the control group (baseline risk) and pooled rate in the treatment group. NNT, number of patients needed to be treated to prevent 1 event.

CONCLUSIONS— Conducting therapeutic trials for a complex and serious problem like diabetic foot infections is difficult, especially when investigating a new adjunctive technology like G-CSF. While our literature search uncovered five randomized trials addressing this issue, it is not surprising that none of the studies enrolled more than 40 patients. Considering the heterogeneous nature of diabetic foot infections and the varied research methods employed, it is difficult to interpret the results of these individual studies. Meta-analysis is the best way to try to determine from the available data if G-CSF therapy can help avert a poor outcome in a diabetic patient with a foot infection.

Our analysis found that adding G-CSF to standard therapy did not appear to benefit the primary outcome of interest, i.e., increasing the likelihood of or hastening the time until resolution of infection. Nor did G-CSF improve the healing of foot wounds. It did, however, have other beneficial effects. Not surprisingly, G-CSF increased the leukocyte count in each of the studies in which this was examined, but the clinical significance of this finding is unknown. Treatment with G-CSF was also associated with a tendency toward a shorter duration of parenteral antibiotic therapy. If true, this could help constrain antibiotic-associated adverse effects, costs, and the development of resistant organisms. More importantly, G-CSF therapy was associated with a statistically significantly reduced risk of requiring lower-extremity amputation as well as other foot infection–related invasive interventions. Because amputations are among the most feared and expensive consequences of diabetic foot infections (50), reducing their incidence would be a

major benefit to diabetic patients and to their health care systems.

This analysis has several limitations.

As with all meta-analyses, our conclusions can only be as accurate as the studies from which they were based. Based on the

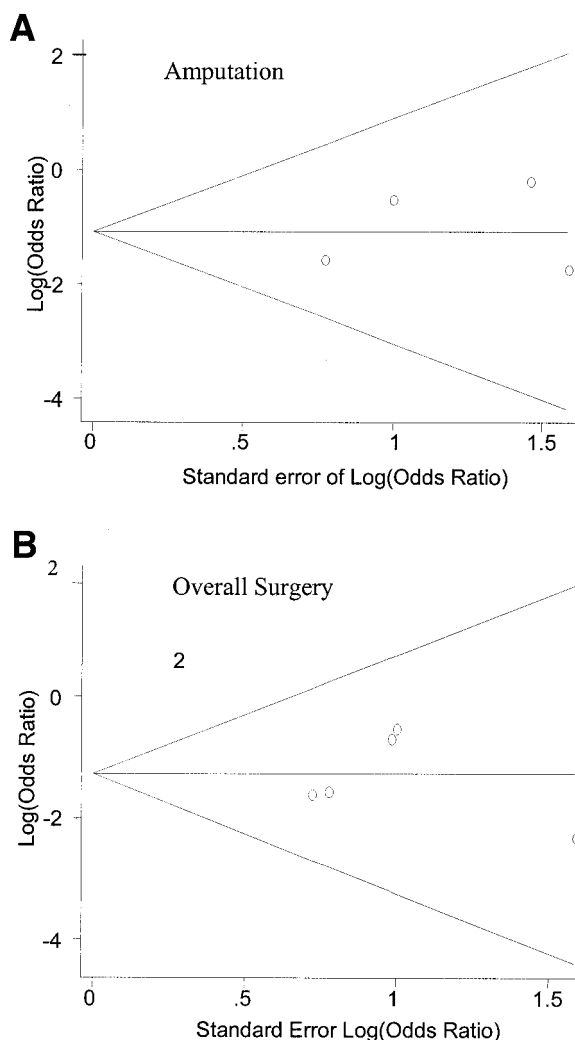


Figure 2— Begg's funnel plot with pseudo 95% CIs for the outcomes "amputation" (A) and "overall surgery" (B). For each study (○), the natural logarithm of the odds ratio is plotted against its SE.

Jadad scores, these G-CSF studies were reasonably well done, but they varied considerably in both design and quality. For instance, the trial by de Lalla et al. (35) included only patients with limb-threatening infections, all of whom had osteomyelitis, while that by Yönm et al. (36) enrolled only patients with relatively mild infections. Most of the studies included patients with foot cellulitis, but Viswanathan et al. (38) excluded patients with foot ulcers, while Kästenbauer et al. (37) enrolled patients with a foot ulcer of Wagner's grade 2 or 3. Similarly, while the antibiotic regimen consisted of a fluoroquinolone combined with either clindamycin or metronidazole in most studies, Gough et al. (8) initiated therapy with four antibiotics, including three β -lactam agents. Moreover, the means of assessing the severity of infection and the study time intervals at which clinical assessments were made varied among studies. Of note is that the studies employed different G-CSF preparations at different dosages by different routes and for different durations. Even the four studies using filgrastim gave products made in different laboratories. There is no way to decide which might be the optimal regimen.

G-CSF is an expensive product that must be administered parenterally. If it does not help cure infections or heal ulcers, one might conclude there is little reason to use it, especially for relatively mild infections. If, however, it can reduce the need for surgical interventions, especially amputations, it may be worth providing. The cost of lower-extremity amputations in persons with diabetes is estimated at >\$30,000 (51,52). The low number of patients who needed to be treated (4.5 and 8.6) that we found to gain these reductions in surgical procedures suggests that this would potentially be a cost-effective therapy. Our analysis of the available data does not support using G-CSF to hasten cure of infection. A formal cost-benefit analysis of these studies could help formulate the most therapeutic strategies. In the meantime, we think clinicians should consider using G-CSF as an adjunct to other appropriate care for a diabetic patient with a foot infection, especially one that may be perceived as limb threatening. The absence of evidence of either heterogeneity among the studies or any publication bias suggests that the conclusions we have drawn are reasonably generalizable and robust.

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