

Bevacizumab Increases Risk for Severe Proteinuria in Cancer Patients

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ABSTRACT

Treatment with the chemotherapeutic agent bevacizumab, a humanized mAb that neutralizes vascular endothelial growth factor, can lead to proteinuria and renal damage. The risk factors and clinical outcomes of renal adverse events are not well understood. We performed a systematic review and meta-analysis of published randomized, controlled trials to assess the overall risk for severe proteinuria with bevacizumab. We analyzed data from 16 studies comprising 12,268 patients with a variety of tumors. The incidence of high-grade (grade 3 or 4) proteinuria with bevacizumab was 2.2% (95% confidence interval [CI] 1.2 to 4.3%). Compared with chemotherapy alone, bevacizumab combined with chemotherapy significantly increased the risk for high-grade proteinuria (relative risk 4.79; 95% CI 2.71 to 8.46) and nephrotic syndrome (relative risk 7.78; 95% CI 1.80 to 33.62); higher dosages of bevacizumab associated with increased risk for proteinuria. Regarding tumor type, renal cell carcinoma associated with the highest risk (cumulative incidence 10.2%). We did not detect a significant difference between platinum- and non-platinum-based concurrent chemotherapy with regard to risk for high-grade proteinuria ($P = 0.39$). In conclusion, the addition of bevacizumab to chemotherapy significantly increases the risk for high-grade proteinuria and nephrotic syndrome.

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Tumor angiogenesis mediated by vascular endothelial growth factor (VEGF) plays a critical role in tumor growth, invasion, and metastasis.^{1–3} Targeting the VEGF signaling pathway has become an important approach to current cancer therapy.^{3,4} Bevacizumab (Avastin; Genentech, South San Francisco, CA), a humanized mAb that neutralizes VEGF, has been approved for the treatment of many advanced cancers, including colorectal cancer (CRC), non-small cell lung cancer (NSCLC), breast cancer, renal cell carcinoma (RCC), and glioblastoma multiforme.⁵

Addition of bevacizumab to chemotherapy increased the risk for proteinuria in comparison with chemotherapy alone, as shown by our meta-analysis based on a total of 1850 patients from seven randomized, controlled trials (RCTs).⁶ We previously demonstrated that relative risks (RRs) for all-grade proteinuria for patients who were administered bevacizumab at 2.5 and 5.0 mg/kg per wk were 1.4

(95% confidence interval [CI] 1.1 to 1.7; $P < 0.001$) and 2.2 (95% CI 1.6 to 2.9; $P < 0.001$), respectively; however, the effect of bevacizumab on the development of severe proteinuria remains unclear.

Severe proteinuria including nephrotic syndrome may cause significant morbidity with a possible consequence of renal failure and fatality. Indeed, among seven of 1459 patients with nephrotic syndrome from bevacizumab treatment in clinical studies, one patient died, one required dialysis, and two had persistent nephrotic proteinuria even after

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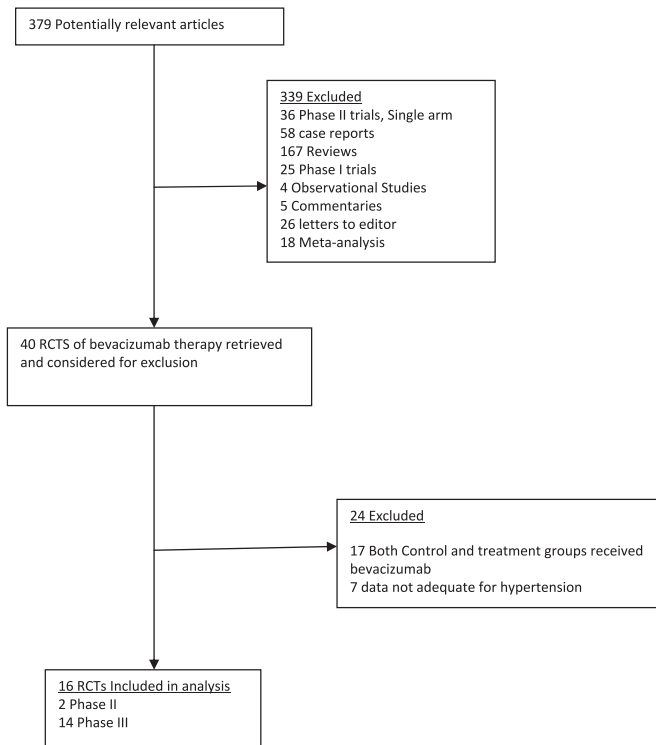


Figure 1. Selection process for RCTs included in the meta-analysis.

discontinuation of bevacizumab.⁵ Severe proteinuria may also limit the use of bevacizumab, thereby compromising its efficacy. It is recommended to suspend bevacizumab temporarily for proteinuria ≥ 2 g/24 h and to discontinue bevacizumab for nephrotic syndrome. The incidences of high-grade proteinuria (grade 3 or above: urine protein ≥ 3.5 g/24 h or dipstick $\geq 4+$ or nephrotic syndrome) in patients who received bevacizumab varied considerably among clinical trials, ranging from 0.6% in a CRC study⁷ to 19.7% in an RCC study.⁸ In addition, risk factors for high-grade proteinuria underlying the variation have not been defined. Because of the limitation with an individual trial in patient number and tumor type, we therefore conducted a systematic review and meta-analysis to evaluate the overall risk and risk factors of high-grade proteinuria with bevacizumab.

RESULTS

Search Results

Our literature search yielded 379 potentially relevant clinical studies of bevacizumab. A total of 16 RCTs were selected for the purpose of analysis (Figure 1). These trials include two phase II and 14 phase III studies, and their characteristics are listed in Table 1. Two RCTs were excluded because of a lack of data for high-grade proteinuria, even though all-grade data were available.^{9,10}

Study Quality

Randomized treatment allocation sequences were generated in all trials. Six trials were double blinded and placebo controlled,^{11–16} two trials had placebo as controls,^{7,17} and the rest of the trials had active treatment control. High-grade proteinuria as the primary outcome of the study was assessed and recorded systematically according to National Cancer Institute's common toxicity criteria version 2 or 3 used in the protocols of these selected clinical trials. Version 2 was used in six trials,^{17–22} version 3 was used in six trials,^{7,8,11,15,16,23} and the rest of the trials did not specify. The quality of all of the trials was acceptable.

Publication Bias

No publication bias was detected for the primary end point of this study (RR of high-grade proteinuria) by Begg test ($P = 0.41$, one-tailed).

Patients

A total of 12,268 patients from 16 RCTs (bevacizumab 6482; control 5786) were included for analysis. Proteinuria was not listed as a baseline characteristic in any of these patients. The baseline Eastern Cooperative Oncology Group status for most of the patients was between 0 and 1. Patients were required to have adequate hepatic, renal, and hematologic function. Baseline renal function was not uniformly defined and included "normal," "adequate," and "serum Cr < 1.8 or 2.0 mg/dl." The exclusion criteria for these studies included the following conditions: Significant cardiovascular disease, peripheral vascular disease, uncontrolled hypertension, serious nonhealing wounds, major surgery within previous 28 days, preexisting bleeding diathesis, brain metastasis, regularly used aspirin (> 325 mg/d) or nonsteroidal anti-inflammatory drugs, pregnant or lactating women, and taking oral or parenteral anticoagulants with the exception of prophylactic anticoagulants to maintain patency of vascular device access. Underlying malignancies included CRC (six studies), NSCLC (two studies), breast cancer (three studies), pancreatic cancer (two studies), RCC (two studies), and malignant mesothelioma (one study). In all trials, patients were randomly assigned to either a control group with chemotherapy alone or a test group with the combination of bevacizumab and chemotherapy. Two studies had a design of three arms: One arm as a control and two arms as a test for different dosage levels of bevacizumab.^{14,15}

Risk for All-Grade Proteinuria

A total of 1355 patients from five RCTs with available data were included for analysis.^{11,16,17,19,21} Among patients who were administered bevacizumab, meta-analysis revealed that the summary event rate of all-grade proteinuria was 13.3% (95% CI 7.7 to 22.1%) using a random-effects model (heterogeneity: $Q = 58.395$, $I^2 = 93.15$, $P < 0.001$). For patients with non-RCC cancers, the summary event rate was 11.9% (95% CI 5.4 to 24.2); there was no significant difference between patients with and without RCC ($P = 0.327$).

Table 1. Characteristics of RCTs included in the meta-analysis

Reference ^a	Trial Phase	No. Enrolled	No. for Analysis	Duration of Follow-up (Months; Median [Range])	Tumor Type	Concurrent Treatment	Bevacizumab Dosage (mg/kg per wk) ^b	CTC Version
Allegra et al., ²³ 2008	3	2710	2647	22.4 (NA)	CRC	Fluorouracil, oxaliplatin, and leucovorin	2.5	3
Escudier et al., ¹¹ 2007	3	649	641	13.3 (0.0 to 25.6)	RCC	Interferon alfa	5.0	3
Giantonio et al., ¹⁸ 2007	3	829	572	28.0 (NA)	CRC	Oxaliplatin, fluorouracil, and leucovorin	5.0	2
Hurwitz et al., ¹⁷ 2004	3	813	790	18.0 (NA)	CRC	Irinotecan, bolus fluorouracil, and leucovorin	2.5	2
Kabbinnar et al., ¹⁹ 2005	2	209	204	14.8 (NA)	CRC	Bolus fluorouracil and leucovorin	2.5	2
Karrison et al., ¹² 2007	2	115	108	15.1 (NA)	Mesothelioma	Cisplatin and gemcitabine	5.0	NA
Kindler et al., ¹³ 2007	3	602	540	11.3 (NA)	Pancreatic cancer	Gemcitabine	5.0	NA
Miles et al., ¹⁴ 2008	3	736	730	10.2 (0.0 to 17.5)	Breast cancer	Docetaxel	2.5 or 5.0	NA
Miller et al., ²¹ 2005	3	462	445	14.8 (NA)	Breast cancer	Capecitabine	5.0	2
Miller et al., ²⁰ 2007	3	722	711	25.9 (NA)	Breast cancer	Paclitaxel	5.0	2
Price et al., ⁴² 2008	3	400	313	NA	CRC	Capecitabine or mitomycin	2.5	NA
Reck et al., ¹⁵ 2009	3	1043	986	NA	NSCLC	Cisplatin and gemcitabine	2.5 or 5.0	3
Rini et al., ⁸ 2008	3	732	715	NA	RCC	Interferon alfa	5.0	3
Saltz et al., ⁷ 2008	3	1401	1369	27.6 (NA)	CRC	Oxaliplatin, fluorouracil, and leucovorin or capecitabine and oxaliplatin	2.5	3
Sandler et al., ²² 2006	3	878	867	19.0 (NA)	NSCLC	Paclitaxel and carboplatin	5.0	2
Van Cutsem et al., ¹⁶ 2009	3	607	583	6.7 (NA)	Pancreatic cancer	Gemcitabine and erlotinib	2.5	3

CTC, Common Terminology Criteria; NA, not available.

^aFunding sources: Seven trials were sponsored by Genentech,^{10,17,19,20,21,43,44} five trials were sponsored by Hoffman Roche,^{7,11,14–16} and six trials were sponsored by the National Cancer Institute and the National Institutes of Health.^{8,9,13,18,20,22,23} One trial was supported by the Mesothelioma Applied Research Foundation.¹² One trial was supported by the Australian Gastrointestinal Trials Group.⁴²

^bDosage was converted from mg/kg schedule.

Table 2. Incidence and RR for high-grade proteinuria with bevacizumab according to dosages and tumor types

Categories	No. of Studies	Bevacizumab (Events/Sample Size)	Control (Events/Sample Size)	Incidence (%; 95% CI)	RR (95% CI)
Overall	16	186/6482	24/5786	2.2 (1.2 to 4.3)	4.79 (2.71 to 8.46)
2.5 mg/kg per wk	7	59/3324	19/3302	1.2 (0.3 to 4.7)	2.62 (1.61 to 4.28)
5.0 mg/kg per wk	10	127/2908	5/2831	3.0 (1.5 to 5.7)	8.56 (4.09 to 17.92)
CRC	6	53/2985	19/2973	1.4 (0.2 to 7.6)	2.52 (1.54 to 4.15)
NSCLC	2	18/1086	0/767	1.6 (0.4 to 6.1)	12.59 (1.67 to 94.80)
RCC	2	78/703	9/653	10.2 (4.3 to 22.4)	48.76 (9.73 to 244.40)
Breast cancer	3	16/1091	0/794	1.1 (0.2 to 5.2)	6.26 (1.11 to 35.46)
Pancreatic cancer	2	18/564	3/544	3.3 (1.9 to 5.5)	4.44 (1.40 to 14.11)
Malignant mesothelioma	1	3/53	1/55	5.7 (1.8 to 16.1)	3.11 (0.33 to 29.0)

The incidence and RR were calculated from the trials included in this study by meta-analysis as described in the Concise Methods section.

We also calculated the RR for all-grade proteinuria from these studies. Using a random-effects model (heterogeneity: $Q = 26.766$, $I^2 = 85.055$, $P < 0.005$), the summary RR with bevacizumab was 2.79 (95% CI 1.31 to 5.95, $P < 0.001$) in comparison with control subjects, suggesting a significantly increased risk for all-grade proteinuria with bevacizumab.

Incidence of High-Grade Proteinuria

High-grade proteinuria is the primary end point of this study, and all of the included RCTs had available data. This encompassed a total of 16 RCTs with 6482 patients who received the treatment of bevacizumab in combination with chemotherapy. Using a random-effects model (heterogeneity: $Q = 252.068$, $I^2 = 94.049$, $P < 0.001$), the summary incidence of high-grade proteinuria was 2.2% (95% CI 1.2 to 4.3%), as shown in Table 2.

Only six studies reported grades 3 and 4 proteinuria separately.^{8,15,18,20,21,22} The incidence of grade 4 proteinuria (nephrotic syndrome) was 0.8% (95% CI 0.4 to 1.8%) among 2333 patients who received bevacizumab, according to the random-effects model (heterogeneity: $Q = 11.3$, $I^2 = 55.752$, $P = 0.046$).

Relative Risk for High-Grade Proteinuria

The observed incidence of high-grade proteinuria in patients who received bevacizumab may be attributable to risk factors such as diabetes, medications, or other underlying diseases. To assess the specific contribution of bevacizumab to the development of high-grade proteinuria and to exclude the influence of confounding factors, we calculated the overall RR for high-grade proteinuria from the trials in which the combination of bevacizumab and chemotherapy was compared with chemotherapy alone. On the basis of these 16 RCTs including 12,268 patients (bevacizumab 6482, control 5786), the summary RR of bevacizumab plus chemotherapy *versus* chemotherapy alone was 4.79 (95% CI 2.71 to 8.46; $P < 0.001$) using a random-effects model (Figure 2A), suggesting that the addition of bevacizumab to chemotherapy significantly increased the risk for high-grade proteinuria as high as 4.79-fold in comparison with chemotherapy alone.

We also determined the RR for nephrotic syndrome with

bevacizumab. RR was not calculable for two trials because no events were reported for either the bevacizumab or the control group.^{18,21} On the basis of four RCTs with available data,^{8,15,20,22} the RR for bevacizumab plus chemotherapy *versus* chemotherapy alone was 7.78 (95% CI 1.80 to 33.62; $P = 0.006$) according to the fixed-effects model (heterogeneity: $Q = 0.48$, $I^2 < 0.001$, $P = 0.923$), suggesting that bevacizumab may significantly increase the risk for nephrotic syndrome.

High-Grade Proteinuria and Bevacizumab Dosage

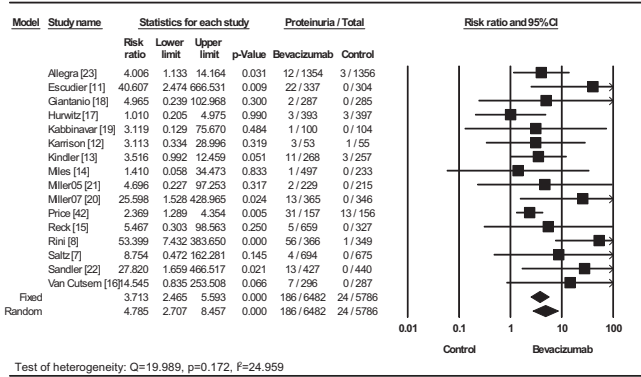
To explore the underlying causes of heterogeneity of the included studies, we examined the relationship between the dosage of bevacizumab and the risk for developing high-grade proteinuria (Figure 2). Among 3324 patients who were treated with low-dosage bevacizumab (2.5 mg/kg per wk) from seven trials, the incidence of high-grade proteinuria was 1.2% (95% CI 0.3 to 4.7%); among 2908 patients who were treated with high-dosage bevacizumab (5.0 mg/kg per wk) from 10 trials, the incidence was 3.0% (95% CI 1.5 to 5.7%). In addition, bevacizumab significantly increased the risk for high-grade proteinuria with an RR of 2.62 (95% CI 1.61 to 4.28; $P < 0.001$) at the low dosage and an RR of 8.56 (95% CI 4.09 to 17.92; $P < 0.001$) at the high dosage. Comparison between the low and high dosages of bevacizumab in RR for high-grade proteinuria showed a significant difference ($P = 0.009$), suggesting that the risk may be dosage dependent.

High-Grade Proteinuria and Tumor Type

The risk for high-grade proteinuria may vary with tumor type (Table 2). There was significant heterogeneity in the incidence of high-grade proteinuria among tumor types ($Q = 82.791$, $P < 0.001$), suggesting that the absolute risk varied with tumor type. The highest incidence was observed for RCC (11.9%; 95% CI 9.7 to 14.7%), and the lowest incidence was seen for NSCLC (2.1%; 95% CI 1.3 to 3.3%). Overall, the incidence of high-grade proteinuria for non-RCC was 3.5% (95% CI 2.9 to 4.5%); there was a significant difference between RCC and non-RCC ($P < 0.001$).

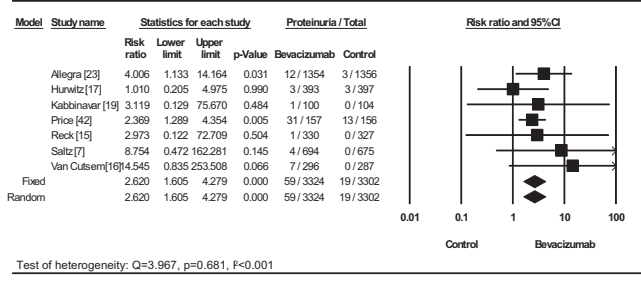
There was a corresponding significant heterogeneity in the RR for high-grade proteinuria among tumors ($P = 0.016$), suggesting RR variation with tumor type. The highest RR was

A RR of high-grade proteinuria with bevacizumab



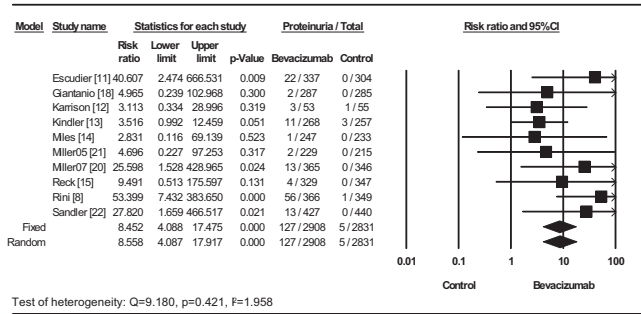
Meta Analysis

B RR of high-grade proteinuria with bevacizumab 2.5 mg/kg/week



Meta Analysis

C RR of high-grade proteinuria with bevacizumab 5 mg/kg/week



Meta Analysis

Figure 2. RR for high-grade proteinuria with bevacizumab compared with control. Both fixed- and random-effects models are used to calculate RR. (A) Overall RR for high-grade proteinuria is reported using the random-effects model. (B and C) Summary RRs for high-grade proteinuria for patients who received bevacizumab at 2.5 mg/kg per wk (B) or 5 mg/kg per wk (C) are reported using the fixed-effects model. The incidence of high-grade proteinuria is compared between patients who were treated with bevacizumab in combination with chemotherapy (bevacizumab) and chemotherapy alone (control) in each study. RR for each study is displayed numerically on the left and graphically on the right. Total events and sample sizes are also displayed for each study. For study name, the first author's name is used to represent each trial. When the same first author is involved in two trials, the publication year is also included to identify the trial. Reference number for each study is listed in brackets. For each trial, the position of the square denotes the value of RR, horizontal lines represent 95% CI, and diamond plot represents overall results of

observed for RCC (48.76; 95% CI 9.73 to 244.40), and the lowest was for CRC (2.52; 95% CI 1.54 to 4.15). Overall, the RR for non-RCC was 3.11 (95% CI 2.03 to 4.75). Comparison between RCC and non-RCC again showed a significant difference ($P = 0.001$). Thus, RCC may be a risk factor for high-grade proteinuria with bevacizumab.

There was also a significant heterogeneity ($Q = 10.203, P = 0.017$) in the incidences of nephrotic syndrome among tumors, suggesting the risk varied with tumor type. The highest risk was observed in patients with RCC (incidence 2.5% [95% CI 1.3 to 4.7%]; RR 18.12 [95% CI 1.06 to 310.15]; $P = 0.046$).

Risk for High-Grade Proteinuria and Platin-Based Chemotherapy

The risk for bevacizumab-associated high-grade proteinuria may vary with concurrent chemotherapeutic agents. To understand the influence of nephrotoxic drugs such as platins (cisplatin, carboplatin, or oxaliplatin) on the occurrence of high-grade proteinuria, we performed a subgroup risk analysis stratified according to platinum- and non-platinum-based chemotherapies. For platinum-based chemotherapy, the RR for high-grade proteinuria with bevacizumab was 5.23 (95% CI 2.17 to 12.57); for non-platinum-based chemotherapy, the RR was 3.38 (95% CI 2.12 to 5.37). There was no significant difference between platinum- and non-platinum-based chemotherapies ($P = 0.39$).

Risk for High-Grade Proteinuria and Clinical Outcome

Because the development of high-grade proteinuria may reflect a biological activity of bevacizumab treatment, we further explored the relationship between high-grade proteinuria and clinical outcome such as progression-free survival (PFS) and overall survival (OS). Bevacizumab significantly increased PFS and OS for patients with NSCLC and CRC and significantly increased PFS for patients with breast cancer and RCC but did not improve PFS or OS for patients with pancreatic cancer and malignant mesothelioma. We performed a meta-regression analysis between the overall risk for high-grade proteinuria and PFS or OS for these studies. No significant correlation was found between RRs for high-grade proteinuria and hazard ratios of PFS ($P = 0.22$). Similarly, we did not find any correlation between RRs for high-grade proteinuria and OS ($P = 0.58$); therefore, the risk for high-grade proteinuria may not be correlated with clinical outcome of bevacizumab-based treatment at the study level.

DISCUSSION

Our study has demonstrated that bevacizumab in combination with chemotherapy was associated with a significantly in-

the included trials. Low-dosage bevacizumab arm of one trial is excluded from RR analysis because of zero events in the treatment and control groups.¹⁴

creased risk for high-grade proteinuria (RR 4.79; 95% CI 2.71 to 8.46; $P < 0.001$) and nephrotic syndrome (RR 7.78; 95% CI: 1.80 to 33.62; $P = 0.006$) in comparison with chemotherapy alone. The clinical significance of severe proteinuria is evident because of its associated renal damage/cardiovascular risk and interruption of bevacizumab therapy; therefore, it is particularly important for physicians and patients to recognize the risk with appropriate vigilance and management.

The development of high-grade proteinuria may be a class effect of VEGF inhibitors. Other VEGF-signaling blockers including axitinib, VEGF Trap, sorafenib, and sunitinib have also been associated with severe proteinuria. The incidence of high-grade proteinuria for axitinib was 5% in a Phase II study involving 60 patients with advanced thyroid cancer.²⁴ In a Phase I trial of 47 patients with a variety of solid tumors, high-grade proteinuria was found to be a dosage-limiting toxicity for VEGF Trap, and the incidence was 4.3%.²⁵ Several cases of nephrotic proteinuria have been reported in patients who were treated with sorafenib or sunitinib.^{26,27}

Proteinuria induced by VEGF inhibition may involve multiple pathways. VEGF is constitutively produced by podocytes with a function of activating VEGF receptor 2 on glomerular capillary endothelial cells, and its inhibition may cause a loss of endothelial fenestrations and podocytes and reduced proliferation of endothelial cells.^{28,29} In addition, VEGF inhibition may cause subacute glomerular thrombotic microangiopathy with features of endotheliosis and membranoproliferative changes, as shown in several biopsy-documented cases.^{30–34} Furthermore, VEGF inhibition may cause hypertension, resulting in an increased intraglomerular pressure and protein filtration; however, hypertension may not play a major role in the development of proteinuria, because the glomerular injury from reduced VEGF expression of podocytes preceded hypertension in a murine conditional knockout model.³⁰

We have identified a relationship between bevacizumab dosage and high-grade proteinuria. Previously, we reported that the risk for all-grade proteinuria may be affected by the dosage of bevacizumab (RR 1.4 for low dosage *versus* 2.2 for high dosage). Consistent with those results, in this study, we showed that RRs for high-grade proteinuria were 2.62 (95% CI 1.61 to 4.28) for the low dosage and 8.56 (95% CI 4.09 to 17.92) for the high dosage of bevacizumab; the difference between the high and low dosages of bevacizumab was significant ($P = 0.009$). After exclusion of RCC trials, RR for high-dosage bevacizumab was 5.41 (95% CI 2.40 to 12.20). The difference between the high and low dosages seems substantial but was NS ($P = 0.135$); therefore, further studies are needed to determine that bevacizumab dosage may be a risk factor for high-grade proteinuria.

Our study demonstrated that the risk for high-grade proteinuria varied with tumor type. Even though all tumor types included in this study had significantly increased risks, patients with RCC seemed to have a particularly high risk, with an incidence of 10.2% (95% CI 4.3 to 22.4%) and an RR of 48.76 (95% CI 9.73 to 244.40). There was a significant difference

between RCC and non-RCC ($P = 0.001$). The higher risk for patients with RCC can be secondary to decreased glomerular filtration because of nephrectomies commonly performed on these patients.³⁵ A decreased GFR may reduce renal clearance and lead to a higher concentration of bevacizumab with a consequently higher risk for proteinuria. It is also possible that the postnephrectomy glomerular hypertrophy of the remaining kidney in patients with RCC may result in more dependence on VEGF to maintain structural integrity than a normal kidney, leading to an increased susceptibility to the anti-VEGF effect of bevacizumab.

Our study showed no significant difference between platinum- and non-platinum-based chemotherapies in the risk for high-grade proteinuria with bevacizumab ($P = 0.99$). This result suggested that bevacizumab-associated proteinuria may not be significantly affected by the concurrent use of nephrotoxic platinum. It may be explained by a different mechanism of action in renal toxicity for bevacizumab (glomerular damage) and platinum (tubular damage). Alternatively, it is possible that our study may not be powered to detect such a difference.

At the study level, we did not find any correlation between high-grade proteinuria and PFS or OS; however, this does not exclude the possibility that a correlation might exist for all-grade proteinuria or at the patient level. Further prospective large trials with standard measurement of proteinuria may be able to address this issue.

Because of the substantial risk for high-grade proteinuria, patients who have cancer and receive bevacizumab need to be monitored before each treatment. This is particularly important for patients who have RCC or are receiving high-dosage bevacizumab, who are at high risk for high-grade proteinuria. Measurement of spot urine protein and creatinine is convenient and reliable³⁶ and may be used to monitor proteinuria instead of cumbersome 24-hour urine protein assay. Preventive measures may include the optimal control of hypertension and the use of angiotensin-converting enzyme inhibitors. Currently, there are no evidence-based guidelines for the management of bevacizumab-associated proteinuria. The packet insert recommends discontinuing bevacizumab for patients with nephrotic syndrome and temporarily suspending bevacizumab for patients with proteinuria ≥ 2 g/24 h (equivalent to urine protein-creatinine ratio ≥ 2) and resumed when it is < 2 g/24 h.⁵ Appropriate measures may include referral to a nephrologist, antihypertensive treatment, and consideration of renal biopsy for worsening proteinuria and renal failure.³⁷ On the basis of our study, dosage reduction may be a reasonable approach to reducing the risk for persistent high-grade proteinuria, particularly when bevacizumab is needed for tumor control.

Our study has several limitations. The findings are influenced by the limitation of individual trials included in the analysis, such as the use of dipstick assessment for proteinuria, no specification of nephrotic syndrome for National Cancer Institute's Common Terminology Criteria grading, and com-

pletteness of follow-up; baseline proteinuria was also not mentioned in these trials, but the risk for high-grade proteinuria was probably very low, because the incidence was 0.4% (95% CI 0.1 to 1.1%) among 5786 control patients. Second, these studies were conducted at various institutions across the United States and hence may contribute to the heterogeneity of reported incidences. We tried to reduce its influence by using a random-effects model to calculate the overall incidence of high-grade proteinuria. Although RRs from all of these studies were similar to each other, our calculation using random- or fixed-effects model may not overcome inherent heterogeneity among source data and may have potential bias regarding the effect of bevacizumab dosage, tumor type, and platinum use. Third, these studies were conducted at major academic institutions for patients with adequate organ function and may not reflect the general patient population in the community or patients with organ dysfunction. Fourth, the study might have a potential observation time bias as a result of prolonged PFS or OS commonly associated with the use of bevacizumab; however, the increased risk for high-grade proteinuria with bevacizumab remained even in pancreatic cancer or mesothelioma trials that did not have increased PFS; the significant risk remained after adjustment for the imbalance in PFS (RR 3.35; 95% CI 1.90 to 5.92) or OS (RR 3.83; 95% CI 2.17 to 6.77), assuming proteinuria occurred evenly over time; in several studies, median onset of high-grade proteinuria was 5.6 months (range 2 weeks to 37 months). Finally, this was a meta-analysis at the study level; therefore, confounding variables at the patient level cannot be assessed properly and incorporated into the analysis.

In summary, our study has shown that the addition of bevacizumab to chemotherapy significantly increased the risk for high-grade proteinuria in patients with cancer. The risk may be modified by bevacizumab dosage and tumor type. It is important for medical oncologists, nephrologists, and patients to recognize the risk with adequate management to prevent renal failure and cardiovascular complications. Future studies are strongly recommended to investigate risk reduction and effective treatment of severe proteinuria.

CONCISE METHODS

Data Source

We conducted an independent review of citations from PubMed between January 1966 and September 2009. Key words included in our search were “bevacizumab,” “Avastin,” and “cancer,” and was limited to “randomized controlled clinical trials.” The search strategy also used text terms such as “proteinuria,” “angiogenesis,” and “vascular endothelial growth factor” to identify relevant information. Abstracts and virtual meeting presentations containing the term “bevacizumab” or “Avastin” from the American Society of Clinical Oncology conferences (<http://www.asco.org/ASCO>) between January 2000 and September 2009 were also referenced to identify relevant clinical trials. We also performed an independent search using the citation da-

Table 3. National Cancer Institute’s toxicity grading criteria versions 2 and 3 for proteinuria

Grade	Version 2 or 3
1	Dipstick 1+ or 0.15 to 1.00 g/24 h
2	Dipstick 2+ to 3+ or 1.0 to 3.5 g/24 h
3	Dipstick 4+ or >3.5 g/24 h
4	Nephrotic syndrome
5	Version 2 none; version 3 death

tabase Web of Science (developed by the Institute for Scientific Information) to ensure that no clinical trials were overlooked. We examined each publication, and only the most recent or complete report of a clinical trial was incorporated when duplicate publications were found. Efforts were made to contact investigators and the manufacturer of bevacizumab when relevant data were unclear. Finally, the updated manufacturer’s package insert of bevacizumab was reviewed to identify pertinent information.⁵

Study Selection

The goal of our study was to assess the specific contribution of bevacizumab to the development of high-grade proteinuria in patients with cancer; therefore, only RCTs with a direct comparison between bevacizumab in combination with chemotherapy (treatment) and chemotherapy alone (control) were incorporated for our analysis. Phase I trials and single-arm Phase II trials were omitted from analysis because of a lack of control subjects. Clinical trials that met the following criteria were included in the meta-analysis: (1) Prospective Phase II or III trials involving patients with cancer; (2) random assignment of participants to bevacizumab in combination with chemotherapy or chemotherapy alone; and (3) available data including event or incidence of grade 3 or 4 proteinuria and sample size. Quality was assessed using criteria including adequate blinding of randomization, completeness of follow-up, and objectivity of outcome measurements as described previously.³⁸

Data Extraction and Clinical End Points

We performed data extraction from these selected trials, including patient characteristics, treatment information, results, and follow-up. Incidences or events of all-grade and high-grade proteinuria and sample sizes were extracted from the safety profile in each trial. Independent data extraction was executed by three reviewers (C.K., L.B., and S.W.). Any discrepancies between reviewers were resolved by consensus. Proteinuria events in these studies were assessed and recorded according to version 2 or 3 of the National Cancer Institute’s Common Terminology Criteria for Adverse Events,³⁹ which have been widely used in cancer clinical trials. These versions are similar in grading proteinuria (Table 3).

Statistical Analysis

We performed all statistical analyses using version 2 of the Comprehensive Meta-Analysis Program (Biostat, Englewood, NJ).⁴⁰ For the calculation of incidence, the number of patients with proteinuria (both high-grade and all-grade) and the number of patients who received bevacizumab were extracted from the selected clinical trials; the proportion of patients with proteinuria and 95% CIs were derived

for each study. For the calculation of RR, patients who were assigned to the combination of bevacizumab and chemotherapy were compared only with those who were assigned to chemotherapy alone in the same trial. To explore a dosage–effect relationship, we further divided bevacizumab therapy into low-dosage (2.5, 5.0, or 7.5 mg/kg per dose per schedule, which is equivalent to a weekly dosage of 2.5 mg/kg) and high-dosage (10 or 15 mg/kg per dose per schedule, which is equivalent to a weekly dosage of 5 mg/dose). The designation of low versus high dosage is relatively arbitrary. We previously showed that all-grade proteinuria may be dosage dependent.⁶

For meta-analysis, both a fixed-effects model (weighted with inverse variance) and a random-effects model were considered.⁴¹ For each meta-analysis, the Cochran Q statistic and I^2 were first calculated to assess the heterogeneity among the proportions of the included trials. For Cochran Q statistic of $P < 0.1$, the assumption of homogeneity was deemed invalid and a random-effects model was reported. The causes of heterogeneity were also explored in this context. Results from the fixed-effects model were reported only when they were not substantially different from the random-effects model. A two-tailed $P < 0.05$ was judged as statistically significant. We used the Begg test to determine the presence of publication bias regarding the primary end point (RR high-grade proteinuria). A two-tailed $P < 0.05$ was considered statistically significant. For correlation between RR for high-grade proteinuria and hazard ratio of PFS or OS, a meta-regression was performed.

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DISCLOSURES

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REFERENCES

- Cavallaro U, Cristofori G: Molecular mechanisms of tumor angiogenesis and tumor progression. *J Neurooncol* 50: 63–70, 2000
- Folkman J: Role of angiogenesis in tumor growth and metastasis. *Semin Oncol* 29: 15–18, 2002
- Hicklin DJ, Ellis LM: Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol* 23: 1011–1027, 2005
- Gerber HP, Ferrara N: Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. *Cancer Res* 65: 671–680, 2005
- Genentech: Avastin (bevacizumab) [Product insert], South San Francisco, Genentech, 2009
- Zhu X, Wu S, Dahut WL, Parikh CR: Risks of proteinuria and hypertension with bevacizumab, an antibody against vascular endothelial growth factor: systematic review and meta-analysis. *Am J Kidney Dis* 49: 186–193, 2007
- Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F, Couture F, Sirzen F, Cassidy J: Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: A randomized phase III study. *J Clin Oncol* 26: 2013–2019, 2008
- Rini BI, Halabi S, Rosenberg JE, Stadler WM, Vaena DA, Ou SS, Archer L, Atkins JN, Picus J, Czaykowski P, Dutcher J, Small EJ: Bevacizumab plus interferon alfa compared with interferon alfa monotherapy in patients with metastatic renal cell carcinoma: CALGB 90206. *J Clin Oncol* 26: 5422–5428, 2008
- Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, Steinberg SM, Chen HX, Rosenberg SA: A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med* 349: 427–434, 2003
- Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, Langer CJ, DeVore RF 3rd, Gaudreault J, Damico LA, Holmgren E, Kabbinavar F: Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* 22: 2184–2191, 2004
- Escudier B, Pluzanska A, Koralewski P, Ravaud A, Bracarda S, Szczylik C, Chevreau C, Filipek M, Melichar B, Bajetta E, Gorbunova V, Bay JO, Bodrogi I, Jagiello-Gruszfeld A, Moore N: Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: A randomized, double-blind phase III trial. *Lancet* 370: 2103–2111, 2007
- Karrison T, Kindler HL, Gandara DR, Lu C, Guterz TL, Nichols K, Chen H, Stadler WM, Vokes EE: Final analysis of a multi-center, double-blind, placebo-controlled, randomized phase II trial of gemcitabine/cisplatin (GC) plus bevacizumab (B) or placebo (P) in patients (pts) with malignant mesothelioma (MM). *ASCO Meeting Abstracts* 25: 7526, 2007
- Kindler HL, Niedzwiecki D, Hollis D, Oraefo E, Schrag D, Hurwitz H, McLeod HL, Mulcahy MF, Schilsky RL, Goldberg RM, Cancer & Leukemia Group B: A double-blind, placebo-controlled, randomized phase III trial of gemcitabine (G) plus bevacizumab (B) versus gemcitabine plus placebo (P) in patients (pts) with advanced pancreatic cancer (PC): A preliminary analysis of Cancer and Leukemia Group B (CALGB). *ASCO Meeting Abstracts* 25: 4508, 2007
- Miles D, Chan A, Romieu G, Dirix LY, Cortes J, Pivrot X, Tomczak P, Taran T, Harbeck N, Steger GG: Randomized, double-blind, placebo-controlled, phase III study of bevacizumab with docetaxel or docetaxel with placebo as first-line therapy for patients with locally recurrent or metastatic breast cancer (mBC): AVADO. *ASCO Meeting Abstracts* 26: LBA1011, 2008
- Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, Leigh N, Mezger J, Archer V, Moore N, Manegold C: Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAIL. *J Clin Oncol* 27: 1227–1234, 2009
- Van Cutsem E, Vervenne WL, Bannouna J, Humblet Y, Gill S, Van Laethem JL, Verslype C, Scheithauer W, Shang A, Cosaert J, Moore MJ: Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J Clin Oncol* 27: 2231–2237, 2009
- Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 350: 2335–2342, 2004
- Giantonio BJ, Catalano PJ, Meropol NJ, O'Dwyer PJ, Mitchell EP,

- Alberts SR, Schwartz MA, Benson AB 3rd: Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: Results from the Eastern Cooperative Oncology Group Study E3200. *J Clin Oncol* 25: 1539–1544, 2007
19. Kabbinavar FF, Schulz J, McCleod M, Patel T, Hamm JT, Hecht JR, Mass R, Perrou B, Nelson B, Novotny WF: Addition of bevacizumab to bolus fluorouracil and leucovorin in first-line metastatic colorectal cancer: Results of a randomized phase II trial. *J Clin Oncol* 23: 3697–3705, 2005
 20. Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, Shenker T, Cella D, Davidson NE: Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med* 357: 2666–2676, 2007
 21. Miller KD, Chap LI, Holmes FA, Cobleigh MA, Marcom PK, Fehrenbacher L, Dickler M, Overmoyer BA, Reimann JD, Sing AP, Langmuir V, Rugo HS: Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. *J Clin Oncol* 23: 792–799, 2005
 22. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilienbaum R, Johnson DH: Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 355: 2542–2550, 2006
 23. Allegra CJ, Yothers G, O'Connell MJ, Sharif S, Colangelo LH, Lopa SH, Petrelli NJ, Goldberg RM, Atkins JN, Seay TE, Fehrenbacher L, O'Reilly S, Chu L, Azar CA, Wolmark N: Initial safety report of NSABP C-08: A randomized phase III study of modified FOLFOX6 with or without bevacizumab for the adjuvant treatment of patients with stage II or III colon cancer. *J Clin Oncol* 27: 3385–3390, 2009
 24. Cohen EE, Rosen LS, Vokes EE, Kies MS, Forastiere AA, Worden FP, Kane MA, Sherman E, Kim S, Bycott P, Tortorici M, Shalinsky DR, Liu KF, Cohen RB: Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: Results from a phase II study. *J Clin Oncol* 26: 4708–4713, 2008
 25. Lockhart AC, Rothenberg ML, Dupont J, Cooper W, Chevalier P, Sternas L, Buzenet G, Koehler E, Sosman JA, Schwartz LH, Gultekin DH, Koutcher JA, Donnelly EF, Andal R, Dancy I, Spriggs DR, Tew WP: Phase I study of intravenous vascular endothelial growth factor trap, aflibercept, in patients with advanced solid tumors. *J Clin Oncol* 28: 207–214, 2010
 26. Jonkers IJ, van Buren M: Nephrotic-range proteinuria in a patient with a renal allograft treated with sorafenib for metastatic renal-cell carcinoma. *Clin Exp Nephrol* 13: 397–401, 2009
 27. Patel TV, Morgan JA, Demetri GD, George S, Maki RG, Quigley M, Humphreys BD: A preeclampsia-like syndrome characterized by reversible hypertension and proteinuria induced by the multitargeted kinase inhibitors sunitinib and sorafenib. *J Natl Cancer Inst* 100: 282–284, 2008
 28. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE: Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest* 111: 707–716, 2003
 29. Schrijvers BF, Flyvbjerg A, De Zeeuw AS: The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. *Kidney Int* 65: 2003–2017, 2004
 30. Eremina V, Jefferson JA, Kowalewska J, Hochster H, Haas M, Weisstuch J, Richardson C, Kopp JB, Kabir MG, Backx PH, Gerber HP, Ferrara N, Barisoni L, Alpers CE, Quaggin SE: VEGF inhibition and renal thrombotic microangiopathy. *N Engl J Med* 358: 1129–1136, 2008
 31. Frangie C, Lefaucheur C, Medioni J, Jacquot C, Hill GS, Nochy D: Renal thrombotic microangiopathy caused by anti-VEGF antibody treatment for metastatic renal-cell carcinoma. *Lancet Oncol* 8: 177–178, 2007
 32. Roncone D, Satoskar A, Nadasdy T, Monk JP, Rovin BH: Proteinuria in a patient receiving anti-VEGF therapy for metastatic renal cell carcinoma. *Nat Clin Pract Nephrol* 3: 287–293, 2007
 33. Stokes MB, Erazo MC, D'Agati VD: Glomerular disease related to anti-VEGF therapy. *Kidney Int* 74: 1487–1491, 2008
 34. Bollee G, Patey N, Cazajous G, Robert C, Goujon JM, Fakhouri F, Bruneval P, Noel LH, Knebelmann B: Thrombotic microangiopathy secondary to VEGF pathway inhibition by sunitinib. *Nephrol Dial Transplant* 24: 682–685, 2009
 35. Schips L, Dalpiaz O, Lipsky K, Langner C, Rehak P, Puerstner P, Pummer K, Zigeuner R: Serum levels of vascular endothelial growth factor (VEGF) and endostatin in renal cell carcinoma patients compared to a control group. *Eur Urol* 51: 168–173, discussion 174, 2007
 36. Ginsberg JM, Chang BS, Matarese RA, Garella S: Use of single voided urine samples to estimate quantitative proteinuria. *N Engl J Med* 309: 1543–1546, 1983
 37. Izzedine H, Massard C, Spano JP, Goldwasser F, Khayat D, Soria JC: VEGF signalling inhibition-induced proteinuria: Mechanisms, significance and management. *Eur J Cancer* 46: 439–448, 2010
 38. Meade MO, Richardson WS: Selecting and appraising studies for a systematic review. *Ann Intern Med* 127: 531–537, 1997
 39. National Cancer Institute: Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Available at: <http://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx>. Accessed September 15, 2009
 40. Biostat I: Comprehensive Meta-analysis. 2006. Available at www.meta-analysis.com. Accessed September 1, 2009
 41. DerSimonian R, Laird N: Meta-analysis in clinical trials. *Control Clin Trials* 7: 177–188, 1986
 42. Price TJ, GebSKI V, van Hazel GA, Robinson BA, Broad A, Ganju V, Cunningham D, Wilson K, Tunney V, Tebbutt NC: International multicentre randomised Phase II/III study of capecitabine (Cap), bevacizumab (Bev) and mitomycin C (MMC) as first-line treatment for metastatic colorectal cancer (mCRC): Final safety analysis of the AGITG MAX trial. *ASCO Meeting Abstracts* 26: 4029, 2008
 43. Herbst RS, O'Neill VJ, Fehrenbacher L, Belani CP, Bonomi PD, Hart L, Melnyk O, Ramies D, Lin M, Sandler A: Phase II study of efficacy and safety of bevacizumab in combination with chemotherapy or erlotinib compared with chemotherapy alone for treatment of recurrent or refractory non small-cell lung cancer. *J Clin Oncol* 25: 4743–4750, 2007
 44. Kabbinavar F, Hurwitz H, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, Griffing S, Bergsland E: Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 21: 60–65, 2003