Review

ONCOLOGY

Endoglin (CD105) as a putative prognostic biomarker for colorectal cancer: a systematic review

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DOI: 10.15386/mpr-2120

Manuscript received: 21.03.2021
Received in revised form: 23.02.2022
Accepted: 10.03.2022

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Abstract

The outcome of colorectal cancer (CRC) can be improved by the identification of prognostic biomarkers. This systematic review of observational cohort and case-control studies was conducted to investigate the role of Endoglin (CD105) in the prognosis of CRC. The databases PubMed, Web of Science, Scopus, and Cochrane CENTRAL were searched to identify the qualified studies using the relevant keywords. After the removal of duplicate articles, the screening was implemented on the titles, abstracts, and potential full-text articles. Afterward, the eligible cohort and case-control studies were identified, and the data were extracted into an Excel datasheet. In total, 11 observational cohort studies and 1 case-control study were identified to be eligible for this systematic review. The majority of the included studies achieved a moderate to high-degree quality according to the Newcastle-Ottawa Scale. Moreover, the eligible studies included a total of 1,400 patients with CRC and mean age of 60 years, the majority of whom were male. Endoglin was observed to be more upregulated in colorectal carcinomas and associated with poor survival outcomes, compared to healthy controls. The levels of Endoglin seem to reflect the degree of cancer invasiveness, therefore predicting dismal prognosis in patients with CRC. Larger and well-designed clinical studies with longer follow-up intervals are needed to investigate the role of Endoglin and its association with cancer metastasis.

Keywords: angiogenesis, biomarker, colorectal cancer, Endoglin (CD105), systematic review, TGF-β family

Introduction

Colorectal cancer (CRC) is considered the third most common cancer worldwide. The overall mortality rate due to CRC was estimated to be 8.9/100.000 in 2018, and the age-standardized incidence rate was high up to 19.7/100.000 [1]. It is predicted that 40-50% of the newly diagnosed CRC patients will have a relapse despite the recent advances in CRC management. Therefore, there is a need for novel biomarkers that would help in the early detection of this disease [2].

Many commonly used clinical and pathological features help predict the prognosis of CRC. These features include the tumor depth of invasion, tumor grade, lymph node status, and presence of metastasis in the liver [3]. However these prognostic parameters often fail to provide accurate prognostic patient stratification. Consequently, novel biomarkers will help predict CRC, thereby fostering better and more accurate treatment protocols for CRC [4].

Patients with less advanced stages of CRC are usually treated by curative resection. Nevertheless, subjects with stages II and III CRC typically require postresection adjuvant chemotherapy [5]. The value of adjuvant chemotherapy is well-established in stage III patients.

However, its role is not consistent in the case of stage II CRC subjects with high-risk features [6]. The better identification of CRC prognostic biomarkers would help stratify subgroups of stage II CRC with a higher possibility of recurrence. Therefore, this strategy has the possibility to decrease the burden of CRC patients [7].

Carcinogenesis is chiefly associated with the processes of apoptosis, formation of new blood vessels, and modifications in cellular proliferation [8]. Several studies have shown an inverse relationship between the apoptotic index (AI) and survival rates [9-11]. Alcaide et al. [12) reported that CRC patients with high AI also had low disease-free and overall survival rates. Angiogenesis plays a vital role not only in CRC growth but also in its progression and metastasis to other organs [13]. Vascular endothelial growth factor (VEGF) is associated with the formation of new blood vessels that characterizes the process of angiogenesis in CRC [14, 15]. The process of angiogenesis can be traced and identified using the panendothelial markers, such as the cluster of differentiation 31, cluster of differentiation 34, and CD105 (Endoglin) [16].

Endoglin, a coreceptor of the transforming growth factor-beta family, has been proved as a marker of neovascularization in solid malignancies, including CRC [17]. Endoglin is excessively expressed in tumor vessels and increases the chances of cancer metastasis [18,19]. Therefore, a series of studies have revealed the role of Endoglin in the prediction of CRC prognosis and treatment [4,16,20-29]. With this background in mind, the present study aimed to evaluate the prognostic significance of Endoglin expression in patients with CRC using a systematic review approach.

Materials and methods

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was strictly followed during the current review [30]. Moreover, all the study steps were performed in strict accordance with the guidelines of the Cochrane Handbook of Systematic Reviews and Meta-analyses [31]. The question behind the research strategy following the Patient, Intervention, Comparison, and Outcome format is whether Endoglin is a prognostic biomarker for CRC patients.

Literature search strategy

The databases PubMed, Web of Science, Scopus, and Cochrane Central Register of Controlled Trials (CENTRAL), were searched since the inception up to November 2020 using the keywords of ("Endoglin" OR "soluble Endoglin") OR "CD105" AND ("Colorectal" OR "colon" OR "colonic" OR "rectal" AND "cancer" OR "carcinoma").

Eligibility criteria and study selection

All the observational cohort studies (i.e., prospective or retrospective] in addition to case-control studies meeting the following criteria were included in this study. The inclusion criteria were localized and locally-advanced stage of CRC, condition (i.e., level of Endoglin marker), outcomes (i.e., angiogenesis biomarker, survival and remission rates), and study design (i.e., observational cohort and case-control studies). The citations of animal studies, studies published in languages other than English, review articles, editorial letters, and proceedings were excluded from the study.

Screening and study selection

The study screening and selection processes were initiated after performing the searching step. All the potential records were screened by two reviewers in two phases, namely title/abstract and full-text screening. Additionally, the reference lists of the included studies were screened for relevant citations. Any article causing disagreements between the two reviewers was reevaluated by the third author and discussed in order to achieve a consensus.

Data extraction

Excel sheets were used to accomplish the data extraction, which was performed by two reviewers. Moreover, the data extraction process was conducted to extract the baseline characteristics of the study participants, risk of bias domains, follow-up period, and survival outcomes. Any disagreement was resolved through a panel discussion.

Risk of bias assessment

The Newcastle-Ottawa Scale (NOS) was used for the assessment of the bias within the included studies. The NOS scores are within the range of 0-8, and the quality appraisal was freely directed by two reviewers. High-quality studies were scored 6 or higher, while low-quality ones were scored below 6. Any disagreement between the examiners was settled by a conversation with the third specialist.

It should be mentioned that all the studies included in this systematic review were scored 6 or above. The observational cohort studies were screened regarding the bias domains of selection (i.e., revising the selection process of the studied cohort), comparability (i.e., inter-group comparability of the cohort group), and outcome (i.e., how was the intended outcome measured? was the follow-up period long enough for outcomes to occur?)

Furthermore, case-control studies were revised in terms of the bias domains of selection (i.e. describing the selection process for case and control groups and establishing a clear definition for cases and controls), comparability (i.e. comparability of the cases and controls based on the design or analysis method), and exposure (i.e. have the cases been already reported with the disease?).

Results

Search results

The primary search using the predefined keywords led to the identification of 93 studies. Duplicate references could be omitted using the Endnote software (version X9); accordingly, 48 articles were obtained. In total, 40 papers were eligible for full-text screening after performing the title and abstract screening step. Subsequently, nine retrospective

cohort studies, two prospective cohort studies, and one case-control study met the inclusion criteria of the current study and were included in the qualitative synthesis. Figure 1 illustrates the process of study selection.

Patient characteristics

A total of 1,400 patients with CRC, mean age of 60 years, 55% male and 45% female, were included in the present systematic review; .The mean follow-up period in the included studies was 25 months, ranging from 1 month to 13 years. Regarding the tumor characteristics, the majority of the CRCs in the current review were classified as stages II and III, and most of them were moderately differentiated. Furthermore, 70% of the lesions were in the colon; however, 30% of them were in the rectum. The size of the lesion was within the range of 5-10 cm in 62% of the included patients.

Histopathology results showed a predominance of adenocarcinoma type over mucinous type. In total, 40% of the colorectal tumors in the current study were classified as stage II disease. The mean overall survival reported was 43 months; nevertheless, the mean progression-free survival was 49 months. The majority of the included studies demonstrated that Endoglin overexpression was associated with a poor prognosis of CRC.

The presence of liver metastasis in the CRC patients was reported in three studies [16,24,29]. In addition, Bal et al. [24] and Saad et al. [21] demonstrated the incidence of distant metastases from the liver in their patients. Likewise, Li et al. [28] and Mitselou et al. [29] estimated the incidence rates of cancer-attributed mortality at 91.25% and 17.3%, respectively.

Out of 12 included studies, eight studies had patients with positive infiltrated lymph nodes [16,21,22,24-26,28,29]. The rate of positive infiltrated lymph nodes was reported within the range of 22-100% in a study performed by Gomceli et al. [26]. Moreover, the patients had lympho-vascular invasion in four studies [4,16,26,29]; nevertheless, two studies only mentioned the occurrence of perineural invasion [4,26]. Table I tabulates the baseline characteristics of the included patients in this systematic review.

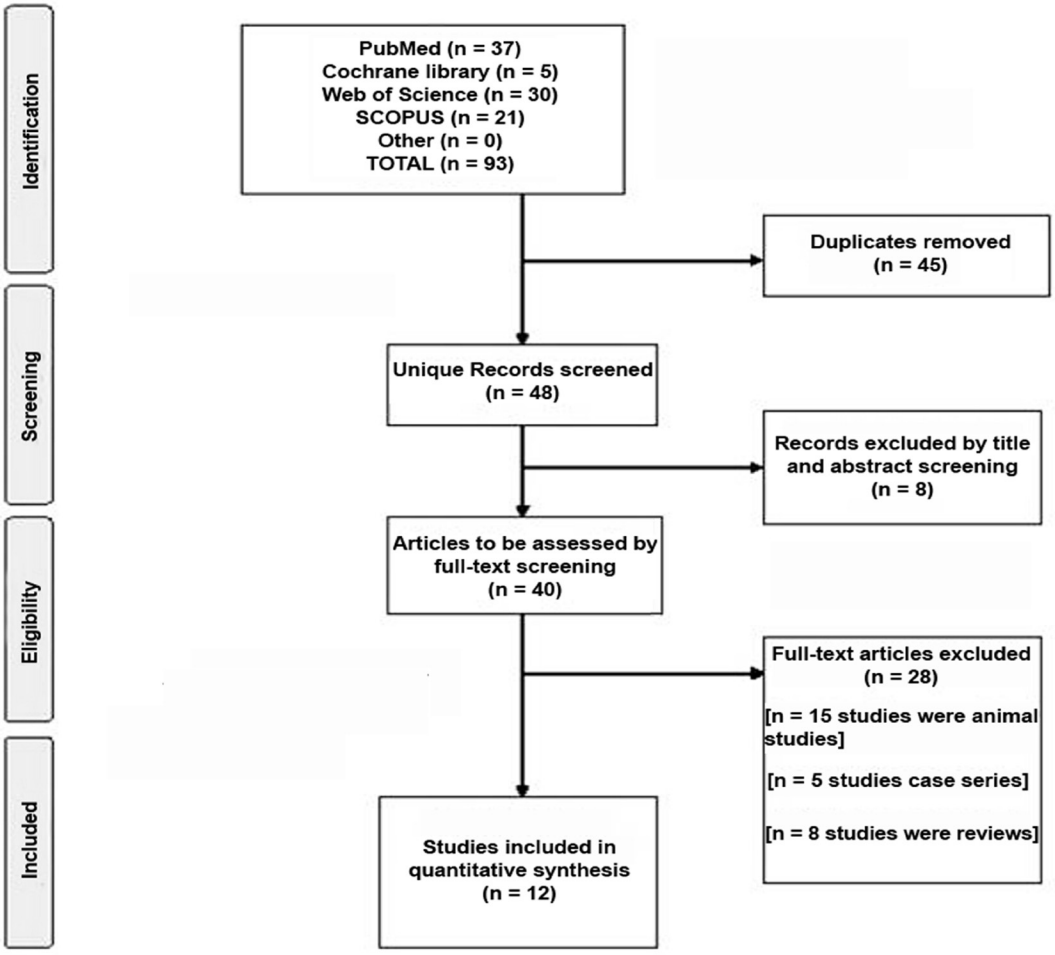

Figure 1. PRISMA flow chart of the study selection.

Table I. Baseline characteristics of included patients.

Reference	Country	Study population	Study design	Age (mean ± standard deviation)	Characteristics of patients	Endoglin evaluation	Overall survival (month)	Progression-free survival (month)	Outcome	Summary of findings
Akagi et al. 2002 [23]	Japan	74	Retrospective cohort	-	Endoscopically resected colorectal adenomas T1, T2 surgically resected colorectal adenocarcinoma	FFPE[2]	-	-	Endoglin value in microvessel quantification in CRC carcinogenesis	Endoglin is an effective marker in the assessment of angiogenesis in CRC development
Bal et al. 2019 [24]	Turkey	47	Prospective cohort	58.5±9.6	Metastatic CRC[1] patients treated with bevacizumab containing chemotherapy	Serum	Mean: 20.8 Standard error: 1.5 95% confidence interval: 17.8-23.7	Mean: 17.5 Standard error: 1.3 95% confidence interval: 14.9-20	Endoglin prognostic value for bevacizumab treatment response	No significance in prediction of treatment response (We checked the data - all are compliant. The information is expressed as non-median averages - median only for follow-up - for OS, PFS mean - even in the Kaplan graphs - tab = mean)
Dassoulas et al. 2010 [25]	Greece	99	Retrospective cohort	69.34±11.59	Surgically treated CRC patients	FFPE	-	-	Association between Endoglin and VEGF[3] expression and correlation with clinico-pathological characteristics and survival	Endoglin and VEGF were positively correlated; increased Endoglin MVD[4] was linked to decreased overall survival
Gomceli et al. 2012 [26]	Turkey	80	Case-control study	62	Surgically treated stage III CRC patients, healthy control group	Serum	-	-	Predictive value of preoperative Endoglin levels for recurrence in stage III CRC	No relation could be observed between Endoglin levels and risk of recurrence nor with any clinico-pathological characteristics
Hawinkels et al. 2010 [27]	Netherlands	48	Retrospective cohort	.	Surgically treated CRC patients, healthy control group	Plasma	-	-	Role of soluble Endoglin in angiogenesis	Soluble Endoglin has antiangiogenic properties and inhibits VEGF induced angiogenesis
Li et al. 2003 [28]	UK	111	Retrospective cohort	69.5	Surgically treated CRC patients	FFPE	Median: 67.2 Range: 3.6-96	-	Prognostic value of Endoglin stained MVD	High Endoglin-stained MVD predicts poor survival
Mitselou et al. 2016 [29]	Greece	69	Retrospective cohort	64.58±7.2	Surgically treated CRC patients	FFPE	-	-	Association between Syndecan-1, E-cadherin/β-catenin, platelet-derived endothelial cell adhesion molecule-1, and Endoglin	Endoglin expression was linked to E-cadherin, β-catenin, and syndecan-1; Endoglin MVD was associated with Dukes' stage, vascular invasion, lymph node and liver metastasis, disease recurrence, and worse survival

Reference	Country	Study population	Study design	Age (mean ± standard deviation)	Characteristics of patients	Endoglin evaluation	Overall survival (month)	Progression-free survival (month)	Outcome	Summary of findings
Mohamed et al. 2019 [16]	Egypt	50	Retrospective cohort	50	Surgically treated CRC patients	FFPE	-	-	Prognostic value of Endoglin MVD	High Endoglin MVD was associated with tumor size, high grade, lymph node invasion, advanced stage, and poor survival
Moreira et al. 2011 [20]	Brazil	60	Retrospective cohort	60	Surgically treated CRC patients	FFPE	-	-	Role of angio- and lymphangio- vascular density in CRC prognosis	No prognostic value for Endoglin MVD
Redondo et al. 2019 [4]	Spain	487	Prospective cohort	68.1	Surgically treated CRC patients	FFPE	Mean: 38.5 95% confidence interval: 36.4-40.6	-	Prognostic value of Endoglin in 2-year survival and clinicopathological features	No association between Endoglin expression and short-term survival or clinicopathological features except [Only MEAN) age
Romani et al. 2006 [22]	Italy	125	Retrospective cohort	65.6	Surgically treated CRC patients	FFPE	Mean: 45.3 Standard deviation: 5.42	-	Role of Endoglin in angiogenesis in CRC metastasis	Endoglin vessel count may detect patients at risk for metastatic disease
Saad et al. 2004 [21]	USA	150	Retrospective cohort	72±9	Surgically treated CRC patients	FFPE	-	-	Comparison of CD31 and Endoglin as a marker for MVD; Endoglin prognostic value in CRC	Endoglin was superior to CD31 in evaluation of MVD; Endoglin MVD was linked to angiolymphatic invasion, lymph node, and liver metastases

1- Colorectal cancer; 2- Formalin-fixed and paraffin-embedded; 3- Vascular endothelial growth factor; 4- Microvascular density

Quality assessment of included studies

The quality of the included studies was moderate up to high according to the NOS assessment tool. The authors' judgment with justifications of the decisions regarding the quality assessment domains is provided in Supplementary file 1.

Role of Endoglin expression in predicting the prognosis of colorectal cancer

Endoglin was upregulated in tumor tissue, compared to normal tissue, as observed in the studies using control groups with benign colorectal masses or healthy controls [20,26,29]. According to Romani et al., [22] there was no correlation between the increase in CD105 levels and change in the site of CRC. Mohamed et al. [16] showed a correlation between high levels of Endoglin and an increase in tumor size. Although this relationship was not proved in the study conducted by Saad et al. [21] regarding the histologic types of colorectal carcinoma, high CD105 levels were associated with mucinous histologic type [16].

In another study, nonmucinous types of CRC had elevated Endoglin levels [18]. High microvascular density (MVD) values given by CD105 were associated with a high incidence of lymph node metastases in two studies [16, 21]. Nonetheless, the MVD values had no significant association with lymph node status in another study [28].

Dassoulas et al. used a cut-off value of 7.3% in the MVD for the evaluation of its prognostic value. In the aforementioned study, a group of CRC patients with MVD of higher than 7.3% was reported with significantly lower overall-survival outcomes than the other group [25]. Similarly, Gomceli et al. [26] observed that the levels of soluble Endoglin were not significantly different between the group with local recurrence of CRC and the group with no local recurrence.

According to a study conducted by Gomceli et al., there was a positive correlation between Dukes' stages and plasma Endoglin level detected by sandwich enzyme-linked immunosorbent assay [26]. Additionally, the MVD assessed by Endoglin levels was significantly associated with a change in Dukes' stages, peritoneal infiltration, venous invasion, and tumor relapse in addition to liver and lymph node metastasis in a study performed by Mitselou et al. [29]. However, Hawinkels et al. revealed no apparent association between serum Endoglin levels and Dukes' stages [27].

Discussion

Our study investigated the value of Endoglin expression in 1,400 CRC patients using a systematic review design, and demonstrated that Endoglin overexpression is associated with poor prognosis in CRC patients. Nine retrospective cohort studies [16,20-23,25,27-29], two prospective cohort studies [4,24], and one case-control study [26] were included. Overall, the quality of evidence was graded as moderate for the current systematic review and ranged from moderate to high for the cohort and case-control studies according to the NOS assessment tool. Based on the obtained results of this study, patients with CRC were observed to have upregulated CD105 expression in the tumor blood vessels. High levels of Endoglin were associated with lymph node metastasis, liver metastasis, and distant metastasis, and in some studies, with tumor size [16,26].

Mohamed et al. [16] demonstrated poor prognosis in CRC patients with high MVD counts, which were determined using CD105. High Endoglin levels were associated with an advanced stage of CRC, worse histologic type, increased nodal metastasis, higher tumor grade, and increased tumor size [16]. In addition, the presence of the metastatic foci in the liver of CRC patients was significantly correlated with the level of Endoglin receptors in a study conducted by Saad et al. [21]. In contrast, Hawinkels et al. [27] showed no statistically significant relationship between CD105 levels in CRC biopsies with tumor size, grade, or histologic grade. Age and gender had no significant effects on the tumor prognosis, nor on cancer behavior.

In a study carried out by Martins et al., targeting Endoglin was suggested in the form of antiangiogenic therapy to combat CRC [32]. Endoglin levels were used for prediction of response to chemotherapeutic agents, radiotherapy, and hormone therapy in laryngeal and breast cancers [33-35]. Uronis et al. observed a statistically significant decrease in the plasma levels of Endoglin while using the chemotherapeutic agent (i.e., bevacizumab) in the treatment of patients with solid tumors [36]. A recent study by Nogués et al. [37] was conducted on 133 CRC patients aiming to assess the value of VEGF and CD105 as diagnostic and prognostic markers for CRC. The results showed that CD105 and VEGF had an essential role in the process of cancer angiogenesis and could be used as biomarkers.

Although most of the studies concerning Endoglin and cancer have focused on its role as a proangiogenic factor and its utility as an MVD marker, Endoglin has not proven clinical utility yet. On the one hand, using Endoglin in the clinics could be facilitated by the easiness of performing immunohistochemical or molecular techniques. On the other hand, technical issues and the use of different anti-endoglin monoclonal antibodies demonstrate differences in reactivity to endothelial cells and this is likely to result in differences in prognostic and therapeutic efficacy [38]. Optimal antibodies should be identified.

Presently, CRC patient prognostic assessment is based on clinicopathological features and focuses on the cancer stage at the time of diagnosis [39]. The main prognostic biomarker used in clinical care is the blood-based carcinoembryonic antigen (CEA) [40].

Mutations in *KRAS/NRAS*, in *BRAF,* along with the mismatch repair gene deficiency microsatellite instability (MSI), are clinically used molecular biomarkers in CRC [41]. Most of these markers might inform clinicians of the overall patient prognosis, but they provide limited information for guiding therapeutic decisions, particularly for early-stage CRC. MSI status is a reliable prognostic marker that can identify "high-risk" early-stage CRC patients who lack benefit from adjuvant chemotherapy. Also, MSI has lately emerged as a predictor of immunotherapy-based treatment sensitivity [42,43]. However, there is a need to find novel predictive biomarkers which may improve these patients' management.

A limitation of our systematic review is the restricted number of studies included. However, this is the reflection of the emerging role of Endoglin as a potential biomarker for CRC management. Other limitations of our systematic review include inconsistences in the design of the evaluated studies including short period of follow-up or lack of serial analysis of CRC patients over time.

Endoglin represents a promising biomarker to predict prognosis of CRC patients and to be associated with cancer metastasis. Well-designed prospective studies to further identify the role of Endoglin in CRC are warranted. While Endoglin has so far not been analyzed as a therapeutic target in CRC patients, our data point to the fact that this might be a promising approach. Additional preclinical and clinical studies will allow a better understanding of the role of CD105 required to improve treatment of CRC patients.

Conclusion

The CD105 levels seem to reflect the degree of cancer invasiveness that can predict the prognosis in patients with colorectal carcinoma. Therefore, larger and well-designed clinical studies with longer follow-up periods to investigate the role of this potential biomarker are required.

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Supplementary file 1. Quality assessment of included studies.

Table SI. Quality assessment results for included case-control studies.

Study ID	4- Gomceli 2012 [26]
Selection (max. 4 *)	
1) Is the case definition adequate?	*
2) Representativeness of the cases	*
3) Selection of controls	*
4) Definition of controls	*
Comparability (max. 2*)	
1) Comparability of cases and controls based on design or analysis	**
Exposure (max. 4*)	
1) Ascertainment of exposure	*
2) Similar method of ascertainment for cases and controls	*
3) Nonresponse rate	
Total	**8**

Table SII. Quality assessment results for included retrospective cohort studies.

Study ID	1-Akagi 2002 [23]	2- Bal 2020 [24]	3- Dassoula 2009 [25]	5- Hawinkels 2010 [27]	6- Li 2003 [28]	7- Mitselou 2016 [29]
Selection (max. 4*)						
Representativeness of exposed cohort	*	*	*	*	*	*
Selection of nonexposed cohort	*	*	*	*	*	*
Ascertainment of exposure	*	*	*	*	*	*
Demonstrating that outcome of interest was not present at initiation of study						
Comparability (max. 2*)						
Comparability of cohorts based on design or analysis	*	*	*	*	*	*
Outcome (max. 5*)						
Assessment of outcome	*	*	*			
Was follow-up long enough for outcomes to occur?		*	*	*	*	*
Adequacy of follow-up of cohorts	*	*	*	*	*	*
Total	**6**	**7**	**7**	**6**	**6**	**6**

Study ID	8- Mohamed 2017 [16]	9- Moreira 2011 [20]	10- Redondo 2019 [4]	11- Romani 2006 [22]	12- Saad 2003 [21]
Selection (max. 4 *)					
Representativeness of exposed cohort	*	*	*	*	*
Selection of nonexposed cohort	*	*	*	*	*
Ascertainment of exposure	*	*	*	*	*
Demonstrating that outcome of interest was not present at initiation of study					
Comparability (max. 2*)					
Comparability of cohorts based on design or analysis		*	*		*
Outcome (max. 5*)					
Assessment of outcome	*	*		*	*
Was follow-up long enough for outcomes to occur?	*	*	*	*	
Adequacy of follow-up of cohorts	*	*	*	*	*
Total	**6**	**7**	**6**	**6**	**6**