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World Workshop on Oral Medicine VII: Biomarkers predicting lymphoma in the salivary glands of patients with Sjögren's syndrome. A systematic review.

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Abstract

Objective: To conduct a systematic review of studies exploring potential biomarkers for development, course and efficacy of treatment of lymphomas in salivary glands of patients with Sjögren's syndrome.

Material and Methods: Eligible studies were identified through a comprehensive search of two databases, i.e. PubMed and EMBASE. Quality of included articles was assessed with the 'Quality In Prognosis Studies' (QUIPS) tool. The 'CHecklist for critical Appraisal and data extraction for systematic Reviews of prediction Modelling Studies' (CHARMS) was used to facilitate data extraction.

Results: Fifty-eight studies met the inclusion criteria. Only one study assessed the progression of lymphoma. Moderate risk of bias was detected in 'outcome measurement', 'study participation' and 'study confounding' domains. Parotid gland enlargement, mixed monoclonal cryoglobulins and low C4 levels represented strongest predictors of lymphoma development. The role of histological biomarkers, and specifically germinal centers, remains controversial. Clinical and methodological heterogeneity across studies precluded conduct of a meta-analysis.

Conclusions: Specific biomarkers in combination with clinical manifestations represent potential candidates for advancing precision medicine approaches to lymphoma prediction in patients with Sjögren's syndrome. Current focus has increasingly been on genetic and epigenetic markers as candidate predictors. Predictive accuracy of key biomarker candidates remains to be tested in well-designed prospectively-followed Sjögren's syndrome cohorts.

Introduction

Sjögren's syndrome (SS) is a common rheumatic disease with an estimated prevalence of 61 cases per 100 000 inhabitants in the general population (Qin *et al*., 2015). SS commonly affects the salivary and lacrimal glands, while the most common symptoms are sensation of dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca) (Vissink *et al*., 2012). Although exact pathogenic mechanism remains to be elucidated, the minor and major salivary glands are characteristically infiltrated by mononuclear lymphoid cells (Kroese *et al*., 2013). Enlargement of major salivary glands, especially the parotid and submandibular gland, is also a common phenomenon. This enlargement is usually bilateral, may be non-painful to slightly tender, as well as intermittent to persistent in nature. An estimated 7.5% of patients with SS develop malignant B-cell lymphoma during the course of their disease, 48-75% of which is of the MALT-type (Sutcliffe *et al*., 1998; Theander *et al*., 2006).

Translation of precision medicine into mainstream clinical care is being prioritized worldwide and is increasingly being advanced as the future paradigm for more effective medical management. Precision medicine, also coined as P4 medicine by Hood and Friend (2011), who characterized it as being 'predictive', 'preventive', 'personalized' and 'participatory', embraces a systems approach to understanding underlying disease pathophysiology coupled with individually-tailored health care informed by an individual's genes, lifestyle and environment (Hodson, 2016). Advancement of precision medicine has remained largely in the increasingly-active research arena which is rapidly expanding the list of candidate biomarkers and definition of clinical phenotypes, with only a few advances of note in the clinical arena. As a recent example, a high pretreatment number of CD20+ B-cells/mm^2 was shown to predict the responsiveness of patients with SS to rituximab treatment, therefore contributing to a more personalized treatment approach (Delli *et al*., 2016).

Since precision medicine approaches to targeting specific molecules in biological pathways continues to successfully make inroads into cancer treatment and improved survival outcomes, research efforts have focused on defining both phenotypic characteristics and molecular signals that underlie emergence of glandular lymphomas that evolve in a subset of at-risk individuals with SS. In order to provide precision medicine to patients with SS, it is also of utmost importance to be able to predict not only the development and course of lymphoma in their

salivary glands but also response to treatment. Such knowledge will allow health care providers to closely monitor high-risk SS patients, intervene as early as possible, and administer a targeted personalized treatment protocol. The growing evidence base continues to report on relevant biomarkers that have been extensively studied to date and continue to remain under investigation. In addition, meta-omics research approaches are rapidly expanding the scope of additional biomarkers which, in combination with clinical phenotypes, may be candidates for translation into the clinical arena in the foreseeable future. Therefore, the aim of this study was to conduct a systematic review in order to identify potential predictive biomarkers for development, course and efficacy of treatment of lymphomas in the salivary glands of patients with SS.

Material and methods

a. Literature search strategy

A systematic literature search of two electronic databases (PubMed and EMBASE) was undertaken to identify publications on biomarkers and other phenotypic characteristics associated with identifying risk for lymphoma detected in salivary glands and course of lymphoma in patients with SS. To insure comprehensiveness of the search, manual review of citations of relevant studies was also undertaken to identify additional relevant publications not identified by the search strategy. No language or temporal restrictions were applied. According to the syntax rules of each database, key words and their combinations were used to identify the studies published prior to June 2018 (Table S1).

b. Study eligibility

Two observers (K.D. and A. Vis.) independently assessed titles and abstracts identified in the initial search. Inclusion criteria were studies examining potential biomarkers predicting lymphoma development or course or efficacy of lymphoma treatment in the salivary glands of patients with SS. Exclusion criteria which applied to title and abstract reviews included: case reports, case series with fewer than five cases, expert opinion publications, letters to the editor, review articles, studies that did not report on potential biomarkers predicting lymphoma development or course or treatment in the salivary glands of patients with SS, and congress abstracts. If the title and abstract provided only limited information, or if eligibility could not be

readily discerned, publications then underwent full text assessment. The results of the assessment were compared, and any disagreement was resolved by consensus of the two reviewers.

Full texts of the included titles and abstracts were independently assessed according to the aforementioned criteria by the same observers. Additionally, the 'QUality In Prognosis Studies' (QUIPS) tool was used to assess the risk of bias of the included studies (Hayden *et al.*, 2013). This tool consists of six domains covering 'study participation', 'study attrition', 'prognostic factor measurement', 'outcome measurement', 'study confounding', and 'statistical analysis and reporting'. After each stage of selection, inter-observer agreement was calculated as Cohen's kappa and percentage of agreement. Studies written in a language in which the assessors were not proficient were translated into English by researchers fluent in both that language and English. Data extraction was performed on study and patient characteristics, and on the validity of biomarkers to predict lymphoma development and efficacy of lymphoma treatment in the salivary glands of patients with SS. The 'CHecklist for critical Appraisal and data extraction for systematic Reviews of prediction Modelling Studies' (CHARMS) was used to facilitate data extraction (Moons *et al*, 2014). The reporting of this study complied with the 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses' (PRISMA) statement (Moher *et al.*, 2009).

c. Statistical analysis

Inter-observer agreement applying Cohen's Kappa and absolute agreement was calculated with IBM Statistic9s 23 (SPSS, Chicago, Illinois, USA). Data were tabulated into a Microsoft Excel spreadsheet and simple descriptive analyses were performed (Microsoft Excel 2010, Redmond Washington, USA).

Results

1. Study identification and selection

A total of 1,086 papers was initially identified. Search results were imported into RefWorks (ProQuest LLC, Ann Arbor, Michigan, USA), compiled and duplicates were removed by the software. The remaining papers were further scanned individually to manually remove undetected duplicates. After excluding duplicates, 814 papers were retrieved and underwent

title and abstract review (Figure 1). Subsequently, 677 titles and abstracts were excluded (a list of all identified papers and excluded papers not presented in this paper can be requested from the corresponding author). Cohen's Kappa agreement was 0.983 and the absolute agreement was 99.6%. Additional manual review of the literature did not identify any articles meeting inclusion criteria. The full text of 137 studies was then screened. Finally, 58 studies were included for quality assessment (Figure 1). Cohen's Kappa and absolute agreement at this stage was 0.803 and 90.4%, respectively. Manual review of citations of relevant studies did not yield any additional eligible studies.

2. Quality assessment of studies

Low risk of bias was observed in 'prognostic factor measurement', 'study attrition' and 'statistical analysis' (84.5%, 55.3% and 50.0% of the included studies, respectively), while moderate risk of bias was identified in 'outcome measures', 'study participation' and 'study confounding' in 91.4%, 75.9% and 65.5% of the studies, respectively. The percentage of studies with high risk of bias was low, and varied across the different domains between 0-12% of the included studies (Figure 2; Supplementary Table 2).

3. Study characteristics

In the 58 studies that were included for quality assessment, SS was diagnosed according to the American European Consensus Group (AECG) Criteria (Vitali *et al.*, 2002), European Community Study Group (ECSG) Criteria (Vitali *et al.*, 1993), or other criteria in 72%, 16% and 12%, respectively. One study used the recently published 2016 American College of Rheumatology-European League Against Rheumatism (ACR-EULAR) criteria (Shiboski *et al.*, 2017). The source of data of the included studies was from cohort (53%), case-control (30%), registry (10%) or other (i.e., case series or unspecified, 7%) studies. In 44% of the included studies, consecutively enrolled patients were included; in 24.5% of the included studies participants were included only if data of interest were available. In 31.5% of the studies the patients' eligibility and recruitment method was not specified. The majority of the studies (53%) were performed at a single institution, while 32% of the studies had multi-center design with the number of participating centers ranging from 2 to 15.

In total, 1,418 patients with SS and lymphoma were investigated. In 77% of studies lymphomas were explicitly defined as non-Hodgkin lymphoma (NHL). Among these, 70% explicitly defined the NHL as B-type. In 65% of the studies, mucosa associated lymphoma tissue (MALT) type was described. Lymphoma type was not specified in 22% of studies. Follow-up of patients was described in 53% of the studies with reported duration of follow-up ranging from 1.9 to 46.6 years. In the majority of studies (94%), the approach to lymphoma diagnosis was not specified nor if it was diagnosed with the same method for all patients.

4. Predictive biomarkers

i. Combination of clinical and serological biomarkers

The majority of studies investigated the predictive value of clinical manifestations *in combination with* serological biomarkers. As shown in Table 1 and regarding the various clinical manifestations assessed, the following were more frequently associated with patients with SS who developed lymphoma: presence of parotid gland enlargement, purpura, peripheral nervous system involvement, splenomegaly, lymphadenopathy, Raynaud's phenomenon, fibromyalgia, high EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI), clinical ESSDAI (clinESSDAI, i.e., ESSDAI without the biological domain), high/high-intermediate international prognostic index (IPI), bone marrow involvement and low grade fever. These clinical manifestations were typically investigated in combination with serological biomarkers, including presence of neutropenia, cryoglobulinemia, hypergammaglobulinemia, hypocomplementemia, (i.e., low C3 levels and/or low C4 levels), leukocytopenia, anemia, monoclonal gammopathy, thrombocytopenia, anti-Ro/SSA or/and anti-La/SSB, rheumatoid factor (RF) positivity, elevated lactic dehydrogenase (LDH) and CD4+ lymphocytopenia. Table 1 summarizes key findings among studies assessing a combination of clinical and serological biomarkers. Parotid gland enlargement in combination with low C4 levels was found to be predictive of lymphoma development in approximately half of the studies (Fragioudaki *et al*., 2016; Ioannidis *et al*., 2002; Ismail *et al*., 2013; Nocturne *et al*., 2016; Risselada *et al*., 2013; Solans-Laque *et al*., 2011).

ii. Serological biomarkers

The predictive value of serological biomarkers was the second most popular studied model with 11 studies retrieved. Serological or laboratory measures under consideration as potential candidate biomarkers associated with emergence of lymphoma in salivary glands among patients with SS included: presence of anemia, anti-SSA and/or anti-SSB positivity, cryoglobulinemia, high levels of Fms-like tyrosine kinase 3 ligand, hypergammaglobulinemia, leukopenia, hypocomplementemia, i.e. low C3 levels and/or low C4 levels, and monoclonal gammopathy (Table 2). Hypocomplementemia and/or cryoglobulinemia, were found to be predictive of lymphoma development in all relevant studies (Brito-Zeron *et al*., 2017, De Vita *et al*., 2012; Kimman *et al*., 2018; Martel *et al*., 2011; Quartuccio *et al*., 2015; Quartuccio *et al*., 2014; Ramos-Casals *et al*., 2005; Ramos-Casals *et al*., 2008; Retamozo *et al*., 2016; Tzioufas *et al*., 1996).

iii. Histological characteristics

One of the most common yet controversial histologic risk factors assessed regarding lymphoma prediction was the presence of germinal centers (GC). Several studies concluded that the presence of GC in diagnostic biopsies was not predictive of MALT lymphoma development in SS patients (Kapsogeorgou *et al*., 2013; Johnsen *et al*., 2014; Haacke *et al*., 2017), while others showed opposite results (Bombardieri *et al*., 2007; Theander *et al*., 2011; Sene *et al*., 2018). Lymphocytic focus score (LFS)≥3 , i.e.≥3 aggregates of 50 or more lymphocytes/4 mm ^{2} of glandular tissue, was found to be able to identify SS patients with an increased risk for lymphoma development (Risselada *et al*., 2014; Risselda *et al*., 2015; Carrubi *et al*., 2015). Similarly, elevated FcRL4+ expression (Haacke *et al*., 2017) and pSTAT-3 expression (Ciccia *et al*., 2015) were reported in diagnostic biopsies of patients who developed MALT lymphoma, while weak or absent A20 staining was observed in the majority of patients with MALT lymphoma (Johnsen *et al*., 2016). Sugai *et al*. (1994) suggested that lymphoma development might be related to suppression of apoptotic death by Bcl-2, while Ussmuller *et al*. (2002) support that myoepithelial sialoadenitis, defined as benign lymphoepithelial lesions, were highly relevant in predicting lymphoma development.

iv. Genetics

Fragkioudaki *et al*. (2017) investigated the prevalence of specific polymorphisms in the methylene tetrahydofolate reductase (*MTHFR)* gene and observed an increased frequency of c. 677C > T TT genotype and T allele, as well as reduced prevalence of the c. 1298A > C C allele in the subset of patients with SS who did not develop MALT lymphoma compared to controls and patients without NHL. Five single nucleotide polymorphisms (SNPs) of the B cell activating factor (*BAFF)* gene (rs1224141, rs12583006, rs9514828, rs1041569 and rs9514827) were evaluated in the study of Nezos *et al*. (2013), where patients with SS at high risk of developing lymphoma were characterized by higher frequency of the minor T allele of the rs9514828 and lower frequencies of the AA genotype of the rs12583006 polymorphism. The rs2230926 exonic variant of the ubiquitin-editing enzyme *TNF AIP3* that regulates nuclear factor kappa B (NF-kB), was associated with an increased risk for lymphoma in the study of Nocturne *et al*. (2013). NF-kB receptor upregulation has been associated with lymphoid tumorogenesis. TNF AIP3 in combination with A20 down-regulates NF-kB activity and acts as a tumor suppressor.

Significantly lower levels of miR200b-5p, a miRNA which regulates expression of autoantibodies directed to intracellular autoantigens, La/SSB, were characterized in SS patients with MALT lymphoma compared with those without ($p<0.05$) (Gourzi *et al*., 2015). Similarly, in the study of Kapsogeorgou *et al*. (2018), miR200b-5p was significantly downregulated in patients with SS who would develop or had NHL, and was able to discriminate this subset ($p<0.0001$) from those without lymphoma or those with non-SS sialadenitis. These investigators reported miR200b-5p as a strong independent predictor of patients who would develop NHL. Regarding methylating enzymes, SS patients with lymphoma were characterized by an intense decrease of methyl CpG-binding protein 2 (MeCP2) and DNA methyltransferase (DNMT) 3B (Mavragani *et al*., 2018). Recently, Vakrakou *et al*. (2018) reported that NLRP3 inflammasome activation and the widespread DNA accumulations in tissues, contributed a key role in NHL development in patients with SS.

v. Chemokines as biomarkers

Barone *et al*. (2008) reported that CXCL12 was principally detected in infiltrated ducts and malignant B-cells in the salivary glands of patients with SS. Additionally, CXCL12 levels were increased in MALT lymphomas and isolated tumor cells, suggesting the direct involvement of CXCL12 in the organization of ectopic reactive lymphoid tissue and its association with the malignant B-cell component and regulation of malignant B-cell survival. Similarly, Nocturne *et al*.

(2015) showed that patients with SS who developed lymphoma had higher levels of serum CXCL13 (a chemokine promoting B-cell chemotaxis that promotes ectopic, B-cell rich, lymphoid tissue formation) than patients with no lymphoma (193.65 pg/ml [IQR=101.71–501.33] vs 108.31 pg/ml [IQR 59.95–197.25]; p=0.006) and also tended to have a higher level of serum CCL11 (139.44 pg/ml [IQR=82.91–177.73] vs 106.66 pg/ml [IQR 70.12–147.19]; p=0.056).

vi. Salivary biomarkers

a. Salivary proteins

Anti-coffilin-1, anti-alpha enolase, anti-Rho GDP-dissociation inhibitor 2 (RGI2) were all found to be over-expressed in patients with SS who developed MALT lymphoma compared with both patients with SS or healthy controls ($p<0.01$; $p<0.001$). The combination of these three auto-antibodies resulted in an AUC value of 0.86 with a 75% sensitivity and 94% specificity in distinguishing SS patients with MALT lymphoma from those without MALT (Cui *et al*., 2017).

b. Parotid scintigraphy

SS patients with class 4 involvement in the salivary glands, i.e. with severe involvement, showing no active concentration of the technetium 99 tracer, were found to have a higher rate of lymphoma development. Additionally, adjusted multivariate Cox regression analysis showed a hazard ratio (HR) of 10.51 (p=0.002) and Kaplan–Meier analysis showed a log-rank of 0.0005 (Ramos-Casals *et al*., 2010).

c. Ultrasound of the major salivary glands (SGUS)

SGUS scores of 2 or 3 (Hocevar *et al*., 2005) were associated with markers of lymphoma. Specifically, GC-like structures in the original salivary gland biopsy findings, CD4 T-cell lymphopenia, and reduced number of memory B-cells in the circulation, immunoglobulin oligo- or monoclonality in serum, the presence of salivary gland swelling, and purpura and skin vasculitis were more frequently observed in patients with SS with several or numerous or confluent rounded hypoechoic lesions compared with patients with a normal SGUS ($p<0.05$) (Theander and Mandl, 2014).

vii. Microbiological biomarkers

Only two studies assessed the potential role of microbiological biomarkers, e.g. viral or bacterial, in the prediction of lymphoma in SS patients. Higher prevalence of *Chlamydophila psittaci* was reported in patients with SS with MALT lymphoma, compared to those with

myoepithelial sialoadenitis or no lymphoproliferative disorder (p=0.02) (Fabris *et al*., 2014). Hirose *et al*. (1999) reported no association between presence of Epstein-Barr virus and lymphoma development in a series of patients with SS.

5. Predictive scoring systems for development of lymphoma in salivary glands of patients with SS

A few studies (n=5) investigated possible predictive scores for lymphoma development by meaningfully combining several risk factors. Table 3 presents a comprehensive overview of these studies. The presence of hypocomplementemia, and specifically of low C4 levels, was a risk factor consistently included in all scoring systems (Baimpa *et al*., 2009 ; Fragioudaki *et al*., 2016 ; Ioannidis *et al*., 2002 ; Quartuccio *et al*., 2014; Solans-Laque *et al*., 2011).

Discussion

In the context of precision medicine, discovery and validation of quantifiable biomarkers defined by omics approaches, in combination with clinical phenotypic definitions associated with disease presentation, is necessary to aid in risk prediction, diagnosis, treatment outcome prediction and assessment of disease progression. Regarding SS, it is of high importance to not only predict which patients with SS are at risk of developing lymphoma, but also to predict site of emergence, since lymphomas could rise either in the salivary glands or stomach or lymph nodes or thymus. This systematic review sought to define the current state of the science relative to application of precision medicine approaches for identification, outcome prediction and management of patients with SS who are at risk for lymphoma emergence in the salivary glands, since lymphomas of the salivary glands occur with the highest frequency (Dong *et al*., 2013), representing-the vast majority of emergent lymphomas observed in SS.

Lymphoma in salivary glands of patients with SS can manifest as completely indolent, but can also be accompanied by severe disease activity, i.e. high ESSDAI, with local or more extensive dissemination. Consequently, treatment may vary from watchful waiting to more therapeutically complex approaches, such as treatment with rituximab, cyclophosphamide and prednisone (R-CP) (Pollard *et al*., 2011). Among foci for advancement of oral precision medicine in the context of SS, is development of the capacity to predict the development of a lymphoma in patients with SS and prognosis associated with the course of lymphoma and efficacy of treatment.

Identification of appropriate biomarkers would advance achievement of these capabilities in the clinical arena. Biomarkers are defined as characteristics that can be objectively measured and evaluated as indicators of normal biological or pathogenic processes, or as indicators of pharmacologic responses to therapeutic interventions (Biomarkers Definitions Working Group, 2001). T

This systematic review delineated 57 different biomarkers in 58 studies as risk factors of lymphoma development in the salivary glands of patients with SS. These could then be further classified in: clinical and histological manifestations, serological or otherwise quantifiable through analysis in a laboratory setting, and by omics approaches including definition of genetic risks, proteomic (expression of chemokines), salivary (proteomic or metabolomic) and microbiological analyses to define potential disease-associated pathogens. The current state of the art suggests that clinical manifestations in combination with serological biomarkers currently represent the most frequently assessed variables in the clinical setting to inform delivery of personalized care, likely because of their ease of applicability. This systematic review determined that the majority of studies focused on identifying biomarkers that predict lymphoma development, while only one study assessed biomarkers predicting the progression of lymphoma (Papageorgiou *et al*., 2015).

Conclusions of available studies regarding histological biomarkers were inconclusive. Studies with central focus on GCs reported contradictory results (Kapsogeorgou *et al*., 2013; Johnsen *et al*., 2014; Haacke *et al*., 2017; Bombardieri *et al*., 2007; Theander *et al*., 2011; Sene *et al*., 2018). This dichotomy could be principally attributed to the considerable variety in histological definitions of GC used in the various studies, as well as to the absence of consensus guidelines to standardize their assessment (Delli *et al*., 2016). Biomarkers could aid in histological definition. Specifically, activation-induced deaminase, an enzyme essential for the function of GC B-cells (Bombardieri *et al*., 2007), the long isoform of CD21 (CD21L); a marker of follicular dendritic cells (Hillen *et al*., 2016), and Bcl-6; a transcription factor expressed at high levels by GC B-cells (Delli *et al*., 2017), have been proposed for definition and identification of GCs. Notably, Nakshbandi *et al*. (2019) recently showed that Bcl-6 is the most appropriate marker for identification of GCs in salivary gland biopsies of SS patients. In contrast to hematoxylin-eosin stain and CD21 immunohistochemistry, Bcl-6 supports unequivocal identification of GCs.

In one-third of the studies, patients' eligibility and recruitment method was not specified, raising concerns about possible selection bias. Additionally, in one quarter of the studies, patients were included only when data of interest were available and no additional analysis was performed to

identify the impact of the missing data. Thus, it is unclear in most of the studies whether data were missing completely at random, at random or not at random. This shortcoming may have resulted in unrepresentative study populations and thus may cause bias in the validity of the biomarkers under investigation (Donders *et al*., 2006).

Lastly, the predictive value of a biomarker, as investigated in a particular study, is applicable to the timeframe in which the recruited patients were followed (Betensky, 2015). In approximately 50% of studies, the exact follow-up period of the patients was not mentioned and the onset of lymphoma in the natural history of SS was not precisely defined. Therefore, it remains unclear how the predictive biomarker should be used in daily practice (Rector *et al*., 2012).

Strengths and limitations

Strengths of the current systematic review were the detailed literature search on the two prevailing databases, i.e. PubMed and EMBASE, without time and language restrictions, assessment of study eligibility by two reviewers, good inter-observer agreement, and application of the QUIPS tool to assess the quality of the studies and utilization of the CHARMS checklist to extract data. The major limitation encountered in interpretation of the outcomes was the high clinical and methodological heterogeneity of the included studies. Specifically, a variation in study populations, study designs, and outcome measures were identified which precluded meta-analysis. Lastly, the fact that studies included often patients with a mix of both salivary and extra-salivary gland lymphomas, cannot exclude the possibility that (some of) the observed predictive biomarkers are not exclusively applicable to salivary lymphomas, but could also be relevant in case of extra-salivary gland lymphomas. Since studies were included only if they reported on ≥5 patients with lymphoma in the salivary glands, the generalizability of the conclusions to the salivary lymphomas is, however, ensured.

Implications and future research

Future studies should comply with the QUIPS and CHARMS guidelines in order to ensure high predictive quality. Particular attention should be paid to the QUIPS domains where moderate risk of bias is observed, viz.:

1) the study sample should adequately represent the SS population, thus a consecutive or random sample of SS patients should be used; a case control design and inappropriate exclusion of patients should be avoided;

2) lymphoma should be diagnosed in a similar manner for all participants, the method used to diagnose lymphoma should be clearly defined and lymphoma should be assessed without knowledge of the candidate predictors;

3) important confounders should be accounted and appropriately measured;

4) researchers should be encouraged to develop predictive models, applying logistic regression analysis, Cox survival analysis, neural networks, or by application of machine learning techniques, whose performance and evaluation should be properly reported. In this respect, Baldini *et al*. (2018) recently showed that with artificial neural network analyses previously hidden formation could potentially be discovered in complex diseases like SS.

The aforementioned features should be stated clearly by authors to avoid potential misinterpretation or poor evaluability. There is a need for an international prospective registry, where patients newly-diagnosed with SS according to the ACR-EULAR criteria are included with subsequent longitudinal follow-up, and collection of structured data at specifically defined time-points.

Conclusion

Rapidly expanding clinical manifestations to support definition of phenotypes and biomarkers with the potential to predict lymphoma in the salivary glands of patients with SS continue to contribute to realization of a precision medicine approach to lymphoma risk prediction in SS. The most commonly used predictive biomarkers and clinical manifestations currently defined for use in clinical practice is documentation of low C4 levels and cryoglobulins in combination with the presence of parotid gland enlargement. The role of histological phenotypes and definition of informative biomarkers remain controversial. Increased research activity was detected with focus on defining specific genetic polymorphisms and useful clinical epigenetic markers. However, due to the high heterogeneity of studies, further research is required to elucidate the predictive value and utility of these biomarkers in a prospectively-followed SS population to evaluate their potential for translation into the clinical setting.

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Conflict of interest

The authors state that they have no conflict of interest.

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Tables:

Table 1: Overview of studies assessing the predictive value of clinical manifestations *in combination with* serological biomarkers.

Authors, year	Participants	Biomarker(s)	Conclusion
Baimpa *et al*., 2009	• 536 SS	• Cryoglobulinemia • Low C4 levels • Lymphadenopathy • Neutropenia • Splenomegaly	• Neutropenia (HR, 8.97; 95% CI, 1.10-73.30; p=0.04), cryoglobulinemia (HR, 2.91; 95% CI, 1.15-6.44; p=0.008), splenomegaly (HR, 3.97; 95% CI, 1.49-10.62; p=0.006), lymphadenopathy (HR, 2.62; 95% CI, 1.15-5.94; p=0.021), and low C4 levels (HR, 3.31; 95% CI, 1.35-8.12; p=0.009) were independent risk factors for the development of lymphoma. • Patients carrying any of these factors had a more than 5.4-fold increased risk of NHL compared to patients with no risk factors.
Baldini *et al*., 2013	• 387 SS • 102 anti-centromere (ACA) positive systemic sclerosis (SSc) • 81 sicca SSc • 41 overlap (SS and SSc)	• Hypergammaglobulinaemia Leukocytopenia • Parotid enlargement Purpura • Peripheral nervous system involvement	• Parotid enlargement, purpura, peripheral nervous system involvement, hypergammaglobulinaemia and leukocytopenia were significant risk factors for non-Hodgkin's lymphoma (p<0.008) in SS patients.
Fragioudaki *et*	• 381 SS without	• Anti-Ro/SS	• Salivary gland enlargement (OR 4.3, 95% 2.0–9.1),

al., 2016	NHL • 73 SS with MALT • 19 SS with (non-MALT) NHL	• Anti-La/SSB positivity • Low C4 levels • Lymphadenopathy • Monoclonal gammopathy • Parotid enlargement • Raynaud phenomenon	lymphadenopathy (OR 4.2, 95% 1.8–9.9), Raynaud phenomenon (OR 2.3, 95% 1.0–5.2), anti-Ro/SS or/and anti-La/SSB positivity (OR 3.8, 95% 1.1–13.4), RF positivity (OR 3.7, 95% 1.4–10.0), monoclonal gammopathy (OR 3.2, 95% 1.0–9.8), and C4 hypocomplementemia (OR 3.0, 95% 1.3–6.8) were identified as independent predictors for NHL development. • The ORs, 95% CIs and p-values for NHL development in the presence of: a. all 7 risk factors were 210.0 (10.0–4412.9), p<0.0001 compared to those with 2 or less risk factors. b. 3 to 6 risk factors were 16.6 (6.5–42.5), p<0.05 in comparison with patients presenting with 2 or less risk factors.
Ioannidis *et al*., 2002	• 723 SS patients	• Low C4 levels • Parotid enlargement • Purpura (palpable)	• Parotid enlargement (HR 5.21, 95% CI 1.76–15.4; p=0.003), palpable purpura (HR 4.16, 95% CI 1.65–10.5; p=0.002), and low C4 levels (HR 2.40, 95% CI 0.99–5.83; p=0.052) were independent predictors of lymphoma or other lymphoproliferative disease. • Patients with at least 1 of these 3 adverse risk factors at baseline had a 9.08-fold higher risk of developing lymphoma or other lymphoproliferative

			disease in the future (95% CI 2.13–38.7) than those who did not.
Ismail *et al*., 2013	• PART I: 8 SS patients with B-cell NHL • PART II: - 50 SS - 25 healthy	• Cryoglobulin positivity • Fibromyalgia • High lactic dehydrogenase (LDH) • Low C3 and C4 levels • Lymphadenopathy • Lymphocytopenia (CD4+) • Parotid enlargement • Purpura	• Significant difference was observed between SS patients with lymphoma and those without lymphoma in the prevalence of parotid enlargement, lymphadenopathy, purpura, fibromyalgia, low C3 and C4, cryoglobulin positivity, and high lactic dehydrogenase (LDH) and CD4+ lymphocytopenia ($p<0.05$).
Kruger & Binder, 1998	• 248 SS	• Anti-SSA • Cryoglobulenaemia • Hypergammaglobulenaemia • Leukopenia, • Neurological involvement • Parotid enlargement • RF (high titre) • Splenomegaly • Vasculitis	• Significant difference between SS patients with lymphoma and those without lymphoma in the prevalence of parotid enlargement, vasculitis, splenomegaly, neurological involvement, hypergammaglobulenemia, cryoglobulenemia, leukopenia, high titre RF, SSA were significantly correlated with the occurrence of lymphoma ($p<0.05$).
Nocturne *et al*., 2016	• PART I: 101 SS patients with lymphoma	• ClinESSDAI score ≥5 • Cryoglobulinemia • ESSDAI	• Model 1: independently associated with development of lymphoma in patients with primary SS were salivary gland enlargement (OR 3.48 95%

	• PART II: - 77 SS patients with lymphoma - 154 SS patients without lymphoma	• low C4 level • lymphopenia • Parotid enlargement • RF	CI 1.50–8.07; p=0.0037), presence of RF (OR 3.04 1. 95%CI 33–6.93; p=0.0083), presence of cryoglobulinemia (OR 3.68 95% CI 1.38–9.83; p=0.0093), low C4 level (OR 3.16 95%CI 1.32–7.55; p=0.0098), and lymphopenia (OR 5.65 95% CI 2.46–12.99; p=0.0001) • Model 2: independently associated with development of lymphoma were ESSDAI (OR 3.84, 95% CI 1.98–7.43; p=0.0001) and RF (OR 3.40 [95% CI 1.71–6.75; =0.0005) • Model 3: independently associated with the development of lymphoma were RF positivity (OR 4.01, 95% CI 1.78–9.00; p=0.0008), presence of cryoglobulinemia (OR 4.07, 95% CI 1.65–10.02; p=0.0023), low C4 (OR 2.33 95% CI 1.05–5.15; p=0.0372) and ClinESSDAI score ≥5 (OR 3.53 95% CI 1.63–7.65; p=0.0014) were independently associated with the development of lymphoma.
Papageorgiou *et al*., 2015	• 77 SS patients with lymphoma	• Total ESSDAI score >10 • International prognostic index (IPI)	• SS patients with lymphoma and high disease activity (total ESSDAI score >10) had greater risk to experience a death (OR=5.241, 95% CI: 1.034–26.568, p=0.045) or an event, i.e. lymphoma relapse, treatment failure, disease progression, histological transformation, (OR=4.317, 95% CI:

			1.146–9.699, p=0.008), and significantly worse event free survival (EFS) and overall survival compared to SS patients with lymphoma and low total ESSDAI (score 10) (EFS: log-rank p=0.001, HR=4.541, 95% CI: 1.772–11.637; OS: log-rank p=0.011, HR=5.946, 95% CI: 1.259–28.077). • Improvement in the total ESSDAI score six months after completion of first-line treatment (delta ESSDAI) among patients who had experienced an event (mean delta ESSDAI±SD: 4.59±1.68) was significantly less than that seen in event-free patients (mean delta ESSDAI±SD: 6.87±3.33) (p=0.005). • In high/high-intermediate international prognostic index (IPI) group of patients, the risk of death was 13.867 times greater (95% CI: 2.656–72.387, p=0.002) and the risk of event was 12.589 times greater (95% CI: 3.911–40.526, p<0.001) compared to low/low-intermediate IPI risk group. SS-associated NHL patients with bone marrow involvement at lymphoma diagnosis had 3.333 times greater risk (95% CI: 1.146–9.699, p=0.027) to experience an event during follow-up.
Risselada *et*	• 195 SS patients	• ESSDAI	• Parotid gland enlargement (OR 2.84) and low C4

al., 2013		• Low C4 levels • Parotid gland enlargement	(OR 7.71) were observed more commonly in SS patients developing NHL. • Presence of IgM kappa clonal components was associated with lymphoma in 64% of cases. • Disease activity (ESSDAI)/severity (cumulative ESSDAI and extraglandular manifestation) scores at first visit could not predict lymphoma development, nor was the pSS disease course significantly worse in patients with NHL.
Solans-Laque *et al*., 2011	• 115 SS patients	• Anemia • Hypergammaglobulinemia • Leukopenia • Low C3 levels • Low C4 levels • Parotid enlargement • Purpura (palpable)	• Unvariate Cox regression analysis identified parotid enlargement (HR 6.75, 95% CI 1.89-23.99), palpable purpura (HR 8.04, 95% CI 2.33-27.67), anemia (HR 3.43, 95% CI 1.04-11.35), leukopenia (HR 8.70, 95% CI 2.38-31.82), lymphocytopenia (HR 16.47, 95% CI 3.45-78.76), hypergammaglobulinemia (HR 4.06, 95% CI 1.06-15.58), low C4 levels (HR 39.70, 95% CI 8.85-126.18), and low C3 levels (HR 36.65, 95% CI 10.65- 116.12) at the time of pSS diagnosis, as significant predictors of lymphoproliferative disease. • The multivariate analysis identified only lymphocytopenia and low C3/C4 levels at pSS diagnosis as independent predictors of lymphoma.
Voulgarelis *et*	• 33 SS patients with	• Anemia	• Lymphadenopathy (65.6%), skin vasculitis (33.3%),

al., 1999	NHL	• Low grade fever • Lymphadenopathy • Lymphopenia • Peripheral nerve involvement • Vasculitis	peripheral nerve involvement (24.2%), low grade fever (25%), anemia (48.1%) and lymphopenia (78.6%) were significantly more frequently observed in SS patients who developed a lymphoma than the general SS population.

Table 2: Overview of studies assessing the predictive role of serological biomarkers alone.

Authors, year	Participants	Biomarker(s)	Conclusion
Brito-Zeron *et al*., 2017	• 1300 SS patients	• Anemia • Cryoglobulinemia • Hypergammaglobulenemia • Low C3 levels • Low C4 levels	• For MALT lymphomas, baseline prognostic factors associated with B-cell MALT lymphomas were cryoglobulins (HR 6.32; $p < 0.001$) and low C3 levels (HR 3.25; p=0.010) • For B-cell non- MALT lymphomas, the prognostic factors included anemia (HR 2.58; p=0.047), monoclonal gammopathy (HR 3.45; p=0.024), cryoglobulins (HR 3.34; p=0.028), and low C4 levels (HR 3.83; p=0.014).
De Vita *et al*., 2012	• 41 SS patients with parotid myoepithelial sialadenitis or B-cell NHL	• Cryoglobulinemia	• Significantly higher prevalence of cryoglobulinemia in SS patients with lymphoma (72.2%) than in SS patients with parotid myoepithelial sialadenitis (72.2% vs 30.4%, p=0.01).
Kimman *et al*., 2018	• 180 SS patients	• Cryoglobulinaemia • Gammaglobulins • IgG • IgM • Low C3 levels	• Cryoglobulins were significantly higher in lymphoma patients compared to non-lymphoma patients (121 ± 250 versus 8 ± 24.9 mg/L for IgG; 231 ± 422 versus13 ± 30 mg/L for IgM; 10 ±

		• Monoclonal bands on protein electrophoresis • Hypocomplementemia	20 versus 1 ± 4 mg/L for IgA in the cryoprecipitate). • Cryoglobulin levels were significantly more increasing (p-values for IgG=0.0007; for IgM=0.0123; and for IgA in the cryoprecipitate <0.0001) in the time period before the lymphoma diagnosis in lymphoma patients compared to non-lymphoma patients • Low C3 (OR 13.9) or C4 (OR 7.1) levels, a decreasing total complement activity (OR 6.6), decreasing gammaglobulins (OR 13.4), a persistent detection of monoclonal bands (OR 14.6) on protein electrophoresis, a low or decreasing serum IgG (OR 18), and decreasing IgM-serum levels (OR 17.7) were significantly associated with lymphoma.
Martel *et al*., 2011	• 445 SS patients	• Cryoglobulinemia	• 9% of SS patients with cryoglobulinemia vs 3% of SS patients without cryoglobulinemia developed lymphoma (p<0.05).

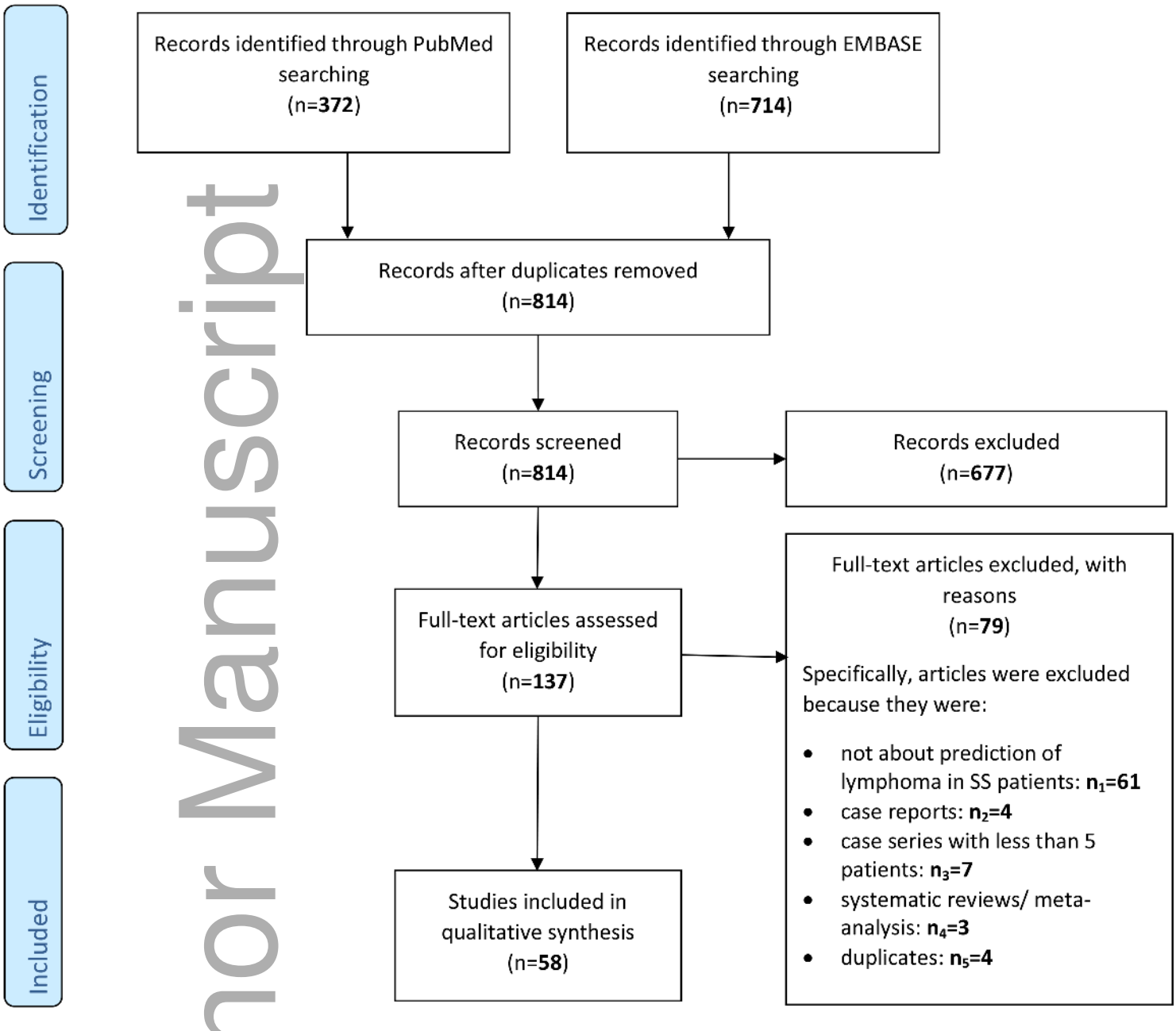
Quartuccio *et al*., 2015	• 548 SS patients	• Anti-SSA • Anti-SSB • Positive histology	• SS patients with a positive histology and SSA/SSB positivity developed lymphoma more frequently compared to the ones with only positive histology (6.4 vs 0.9%, p=0.002) and absence of SSA/SSB.
Quartuccio *et al*., 2014	• 40 SS patients with NHL • 17 SS patients with cryoglobulinemic vasculitis • 180 SS patients with salivary gland swelling • 424 SS patients without NHL or prelymphomatous conditions	• Anti-SSB • Cryoglobulinemia • Leukopenia • Low C4	• Positive serum cryoglobulins [relative-risk ratio (RRR) 6.8, 95% CI 2.1e22.1], low C4 (RRR 8.3, 95% CI 3.6-19.2), anti-La (RRR 5.2, 95% CI 2.3-11.9), and leukopenia (RRR 3.3, 95% CI 1.5-7.05) were the selected variables, by multinomial logistic analyses, that distinguished SS patients with NHL from control group. • A score 2 (i.e., the positivity of at least 2 biomarkers), showed a sensitivity for lymphoma of 71.8% (CI 55.1-85.0) and specificity of 79.0% (CI 72.1-84.9).
Ramos-Casals *et al*., 2005	• 336 patients with SS	• Low C3 levels • Low C4 levels	• SS patients with low C4 levels showed a higher prevalence of lymphoma (10 vs 2%, p=0.013); • SS patients with low C3 levels showed

			a higher prevalence of lymphoma (10 vs 2%, p=0.017).
Ramos-Casals *et al*., 2008	• 1010 patients with SS	• Hypocomplemetemia	• SS patients with hypocomplemetemia had a higher frequency of lymphoma (p=0.01).
Retamozo *et al*., 2016	• 515 patients with SS	• Cryoglobulinemia • Vasculitis (cryoglobulinemic)	• Compared with patients without cryoglobulins, patients with cryoglobulins who fulfilled [hazard ratio (HR)=7.47, 95% CI: 3.38, 16.53] and did not fulfil (HR=2.56, 95% CI: 1.03, 6.35) cryoglobulinemic vasculitis criteria both showed a higher risk of B-cell lymphoma in the univariate analysis, but not in the multivariate models.
Tobon *et al*., 2013	• 369 patients with SS • 50 healthy controls	• Fms-like tyrosine kinase 3 ligand	• Higher levels of Fms-like tyrosine kinase 3 ligand were significantly associated with a history of lymphoma (p=0.0001) in SS patients.
Tzioufas *et al*., 1996	• 103 patients with SS	• Cryoglobulinemia	• Cryoglobulinemia was found as the predominant factor (r=0.421, p=0.0009) for lymphoma development.

Table 3: Scoring systems for predicting lymphoma development in the salivary glands of patients with SS.

Authors, year	**Risk factors**	**Prognostic classification**	**Probability or risk of developing lymphoma per group**
Baimpa *et al*., 2009	1. Cryoglobulinemia 2. Low C4 levels 3. Neutropenia 4. Splenomegly	**Group A**: Low risk if presence of 0 risk factors **Group B:** High risk if presence of ≥1 risk factors	**Group A**: 3.6% probability **Group B:** 20.6% probability
Fragioudaki *et al*., 2016	1. Anti-Ro/SS 2. Anti-La/SSB positivity 3. Low C4 levels 4. Lymphadenopathy 5. Monoclonal gammopathy 6. Parotid enlargement 7. Raynaud's phenomenon	**Group A:** presence of ≤2 risk factors **Group B**: presence of 3-6 risk factors **Group C**: presence of all 7 risk factors	**Group A:** 3.8% probability **Group B**: 39.9% probability **Group C**: 100% probability
Ioannidis *et al*., 2002	1. Low C4 levels 2. Palpable purpura 3. Parotid enlargement	**Group A:** Low risk if presence of 0 risk factors **Group B:** High risk if presence of ≥1 risk factors	**Group A**: reference group **Group B:** 9.08-fold higher risk than Group A
Quartuccio *et al*., 2014	1. Anti-SSB 2. Cryoglobulinemia 3. Leukopenia 4. Low C4 levels	**Group A**: Presence of <2 risk factors **Group B:** presence of ≥2 risk factors	**Group A**: reference group **Group B:** 10-fold risk than Group A

Solans-Laque *et al*., 2011	1. Lymphocytopenia 2. Hypocomplementemia	**Group A**: Low risk if presence of 0 risk factors **Group B:** High risk if presence of ≥ 1 risk factors	**Group A**: reference group **Group B:** significantly higher risk than Group A (no exact numbers are given)



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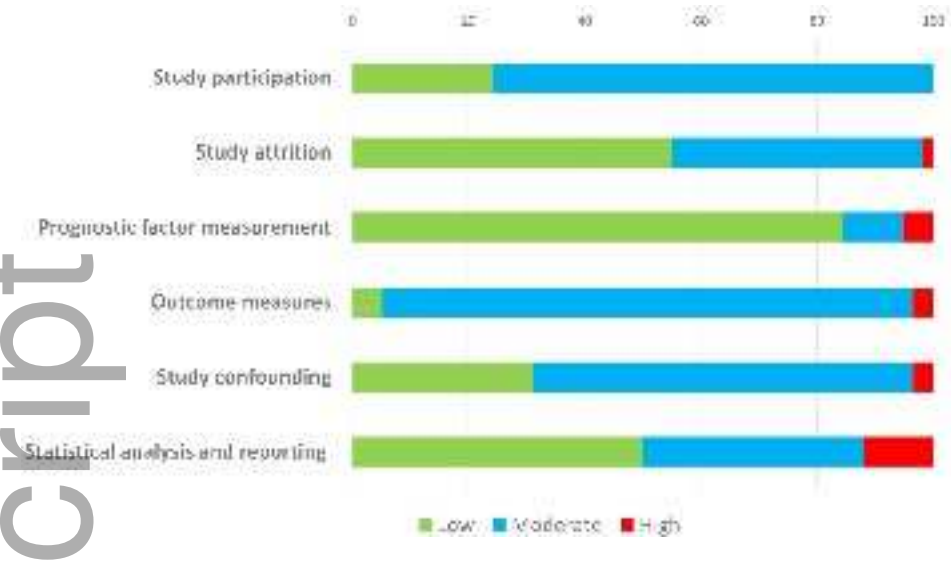

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