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Title: Neuroendocrine cells in prostate cancer correlate with poor outcomes: A systematic review and meta-analysis.

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Abstract

Objectives: To determine the prognostic utility of reporting neuroendocrine staining at prostate cancer diagnosis, we performed a systematic review and meta-analysis. Specifically, we aimed to understand the variability in reporting of neuroendocrine staining, and determine whether different reporting approaches impact the correlation between staining and patient outcome.

Methods: Medical databases were searched to identify studies where adenocarcinoma specimens were stained with any of four neuroendocrine markers: Chromogranin A (CgA), Neuron Specific Enolase (NSE), synaptophysin and CD56. Prevalence of neuroendocrine staining and correlation to patient outcomes were analysed using a random-effects model. All statistical tests were two-sided.

Results: Sixty-two studies spanning 7,616 patients were analysed. The pooled prevalence for the most common marker, CgA (41%), was similar to NSE (39%) and higher than synaptophysin (31%). Prevalence of CgA staining was significantly influenced by reporting criteria, where objective thresholds reduced the variation in prevalence to 26%. No correlation was found between CgA prevalence and tumour grade. Patients positive for CgA staining using objective criteria had more rapid biochemical progression (hazard ratio 1.98 (95% CI = 1.49 to 2.65)) and poorer prostate cancer-specific survival (hazard ratio: 7.03 (95% CI = 2.55 to 19.39)) compared to negative patients, even among those with low risk cancers.

Conclusion: Discrepancies in the reported prevalence of neuroendocrine cells in adenocarcinoma are driven by the inconsistent scoring criteria. This study unequivocally

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demonstrates that when neuroendocrine cell staining is assessed with objective criteria it identifies patients with poor clinical outcomes. Future studies need to determine the exact quantifiable thresholds to report neuroendocrine cell staining, to identify patients at higher risk of progression.

Introduction

The clinical significance of neuroendocrine differentiation at prostate cancer diagnosis remains uncertain. For many decades, immunohistochemistry has been used to reveal foci of neuroendocrine cells in otherwise conventional prostate adenocarcinomas, a pathological variant known as adenocarcinoma with neuroendocrine differentiation [1]. Reports suggest anywhere from 10% - 100% of patients harbour these lesions in diagnostic specimens [2, 3]. The aetiology of neuroendocrine cells in prostate adenocarcinoma has been intensely investigated, yet the significance of these cells remains unresolved. Similarly, any benefit in detecting neuroendocrine cells to provide additional prognostic information to reporting tumour grade and stage currently remains inconclusive [3, 4]. As such, there is currently no clinical recommendation to perform immunohistochemistry with neuroendocrine markers on diagnostic biopsies or prostatectomies, to assist with the risk classification of prostate adenocarcinomas [3].

However, the value in defining the prognostic potential of reporting neuroendocrine staining should be revisited given our contemporary understanding of neuroendocrine differentiation in prostate cancer. While once considered rare, small cell or large cell neuroendocrine tumours are now detected more frequently, specifically in men who have progressed to castration-resistant prostate cancer [5-7]. Detection of these neuroendocrine tumours is important for clinical decision making, as they are highly aggressive and inherently resistant to androgen signalling inhibitors used for treating advanced prostate cancer [8]. Despite these recent observations demonstrating the significance of detecting neuroendocrine tumours later in disease progression, there has been no reassessment as to whether detection of neuroendocrine cells in diagnostic adenocarcinoma specimens could also be useful in the clinic. This could be particularly insightful for men with lower grade tumours where even a small percentage of neuroendocrine cells could be prognostic.

The aim of this study was to perform a systematic review and meta-analyses of the literature, to understand the variation in the reporting of neuroendocrine staining and determine the

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influence of reporting neuroendocrine staining at diagnosis on patient outcomes. Here we show that objective criteria used to reporting the presence of neuroendocrine cells is prognostic for biochemical progression prostate cancer-specific survival, even for low risk cancers.

Methods

Search strategy and selection criteria

The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines were used in this systematic review [9]. This review was registered in the international prospective register of systematic reviews (PROSPERO ID CRD42018117906).

An electronic search was conducted using the following databases: Medline, Embase, Biosis Previews, Cochrane Central Register of Controlled Trials and Scopus. The databases were searched from inception to 9th July 2021. The initial search strategy was piloted in Medline and adapted to the search terms of other databases. The full search strategy is provided in Figure S1.

Title and abstract screening, followed by fulltext review of potentially eligible articles was carried out by two independent reviewers (A.K and R.T) using Covidence software (Veritas Health Innovation, Melbourne, Australia), a web-based tool that facilitates the screening of articles and data extraction. Any discrepancies were resolved by discussion. As the primary objective was to assess the reporting of adenocarcinoma with neuroendocrine differentiation through neuroendocrine biomarker staining in patients, we considered articles for inclusion if they included prospective or retrospective cohorts, cross sectional and/or descriptive studies. Case reports, review articles, meeting abstracts and non-English publications were excluded.

The initial assessment identified studies where neuroendocrine marker staining was performed on prostate cancer samples from hormone naïve men. We restricted our analysis to studies which used at least one of four neuroendocrine biomarkers, including two that are currently used to detect neuroendocrine differentiation in prostate cancer (Chromogranin A (CgA), Synaptophysin (Syn)), and two other biomarkers which have been used historically in prostate cancer studies to detect neuroendocrine cells (Neuron specific enolase (NSE) and CD56)[3, 10, 11]. We included any studies, which analysed these four biomarkers either separately or in combination with each other. Papers were eligible if the proportion of

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patients who stained positive for neuroendocrine markers was reported. In order to focus on neuroendocrine staining in hormone naive prostate cancer, several exclusion criteria were set including 1) studies assessing de novo neuroendocrine prostate cancer; 2) studies with less than ten patients; 3) studies that had mixed hormone naïve and castration-resistant patients. Additionally, when studies were identified with overlapping patients, the study, and/or cohort with the smallest sample size, was excluded. For studies which had data for two tissue types from the same patient (i.e. biopsy and matching radical), we restricted our analyses to the radical prostatectomy samples.

The primary outcome measure was prevalence of positive staining with neuroendocrine marker immunohistochemistry in hormone naive prostate cancer. Secondary outcome measures included correlation of neuroendocrine staining with Gleason score, biochemical progression, clinical progression, prostate cancer-specific survival and overall survival.

Quality Assessment

Quality assessment was conducted by A.K. and R.T. using the REporting recommendations for tumor MARKer prognostic studies criteria (REMARK: Table S1)[12]. REMARK is a gold standard reporting guideline for prognostic biomarker studies and was adapted to assess the quality of papers included in the meta-analysis (Table S1). For this study, 13 REMARK criteria were used to assess studies with prevalence only (no clinical follow-up) and 19 REMARK criteria were used for studies with clinical follow-up. For each criterion, a maximum mark of one was allocated for complete reporting, half a mark was given for partial reporting and no marks were allocated for absence of reporting. The methodological quality of each study was determined to be higher or lower than the possible mean score for studies with prevalence only (6.5/13) or studies with clinical follow-up (9.5/19).

Data extraction

Data extraction was carried out by two independent reviewers (A.K and R.T), with discrepancies resolved by discussion. The following parameters and outcomes were extracted from the included studies: first author, country of study, study design, prostate cancer specimen source, tumour grade, neuroendocrine markers used, criteria for reporting neuroendocrine staining, proportion of samples reported positive for staining.

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The included studies used different criteria to report the prevalence of neuroendocrine staining, and often included multiple cut-offs in the analyses. In this systematic review, we selected a single criterion from each study for analysis, being the criterion used to categorise patients for correlation analyses.

Subsequently, when clinical parameters were available, a correlation analysis was performed to assess the prevalence of neuroendocrine staining with tumour grade, biochemical recurrence (determined by PSA level), clinical progression (determined by PSA level and/or detection of metastases), overall survival and prostate cancer-specific survival.

For correlation to tumour grade, patients were categorised according to those who were Gleason score ≤7 and Gleason score ≥8. For clinical outcomes, we extracted hazard ratios (HRs), relative risk (RR) and 95% CIs. When a RR was not provided, it was extracted from the Kaplan-Meier curve using the Digitize it software (http://www.digitizeit.de/) or from data provided as raw values or percentages where available [13].

Statistical analysis

A meta-analysis of the prevalence of neuroendocrine staining was performed on Stata version 16 (StataCorp LLC, Texas, USA). All statistical tests were two-sided and exact P values are provided where possible. A meta-analysis of proportions was performed to determine the prevalence of neuroendocrine marker staining in hormone naïve prostate cancer according to the four neuroendocrine markers CgA, Syn, NSE and CD56. Meta-analysis of proportions and respective 95% confidence intervals (CIs) were analysed using a REstricted Maximum Likelihood (REML) random-effects model. REML considers the degrees of freedom when estimating variance components, which is more suitable for performing meta-analysis models with fewer studies [14]. Heterogeneity was determined using the I^2 statistic, which examines the percentage of variation across studies that is due to heterogeneity rather than chance [15].

Subgroup and multivariable meta-regression analyses were performed to assess possible sources of heterogeneity on the reporting of neuroendocrine prevalence. Subgroup and meta-regression analyses was restricted to studies using CgA. If studies had more than one cohort of patients for the multivariable analyses, only the prostatectomy cohort was included. This was to avoid duplicating and inflating shared study characteristics of these cohorts.

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Subgroup analyses were performed on the following variables: tissue type, criteria for reporting neuroendocrine staining, antibody type, study design and study quality (REMARK score). Tissue type was categorised according to whether tumours were obtained at prostatectomy, prostatic biopsy, transurethral resection of the prostate (TURP), prostate tumours processed as a tissue microarray (TMA) or from metastatic sites (metastases). Studies were allocated to one of three categories in order to investigate their reporting criteria for neuroendocrine staining. Studies that use quantifiable thresholds were categorised as "objective criteria". Studies using objective criteria were further subgrouped according to specific objective thresholds, where the comparison was restricted to thresholds that were used in at least two studies. The other two categories were 1) studies that did not clearly define reporting criteria or used qualitative and non-reproducible criteria, categorised as "subjective criteria", and 2) studies that explicitly compared negative staining to positive staining, categorised as "any positive cells". Antibody type was categorised according to whether the CgA antibody used in each study was monoclonal, polyclonal, or unknown. Studies were allocated into four categories according to their study design: Cross-sectional, descriptive, retrospective cohort and prospective cohort studies. The impact of study quality was assessed by categorising studies according to whether they fell above or below the median REMARK score.

Multi-variable meta-regression analyses considered the additional impact of samples size (handled as a continuous variable) in addition to the above subgroup strata. Significance level for subgroup difference and meta-regression was set at 0.05.

To assess the association between neuroendocrine staining and tumour grade and patient outcomes, we first separated studies based on their criteria for reporting neuroendocrine staining before performing meta- or subgroup analyses. For tumour grade, we compared prevalence of CgA in tumours categorised according to lower Gleason score ≤ 7 and higher Gleason score ≥ 8. For patient outcomes, we assessed log-transformed HR and RR with respective 95% CIs of patients with the CgA staining and biochemical recurrence, clinical progression, overall- and prostate cancer-specific survival using the REML random-effects model.

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Both graphical and statistical methods were used to assess publication bias on all studies including CgA staining. The sole inclusion of larger studies would lead to clustering around the true prevalence on the plot, indicating the bias of excluded smaller studies. A random scatter of prevalence reported by included studies would confirm the inclusion of both small and large studies. A funnel plot was generated to assess the distribution of prevalence across the published studies. Funnel plot symmetry and small study effects were evaluated through Egger's and Begg's tests [16, 17]. Trim and fill method was applied to simulate a pooled prevalence value that would be reported in the absence of publication bias [18]. To indicate any missing studies, the simulated value of 'observed + imputed' effect size would differ to the 'observed' effect size of the current included studies.

Results

A total of 4,982 studies were identified from our search strategy. Of these studies, 4,743 were not relevant to the research question upon screening of title and abstract, and a further 177 studies screened by full-text were excluded as they did not meet the inclusion criteria. In total, 62 studies met the eligibility criteria for data extraction and analysis (Figure 1).

The 62 eligible studies included 7,616 prostate tumour specimens that were stained for at least one of the four neuroendocrine markers: CgA, Syn, NSE and CD56. Thirty-three of these studies correlated the presence of neuroendocrine staining to patient outcomes such as biochemical recurrence, clinical progression, overall survival and prostate cancer-specific survival. The characteristics of the included studies are summarised in Table 1 [19-80] and Table S2.

Risk of bias

The included studies were scored against the REMARK criteria. Overall the majority of studies were of moderate quality, with 51/62 studies meeting at least 50% of the REMARK criteria (Table S1). Studies that assessed prevalence of neuroendocrine staining had a median score of 8/13, whereas studies that also included clinical follow-up had a median score of 11/19 (Figure S2).

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Variation in the prevalence of neuroendocrine staining is independent of the neuroendocrine marker

The majority of studies used CgA to detect neuroendocrine cells (N=53), with a pooled prevalence of 41%. There was considerable heterogeneity in the reported prevalence of neuroendocrine staining across these studies (I^2 = 98.25%), with the prevalence of individual analyses ranging from 4-100% (Figure 2A). The pooled prevalence for analyses using NSE and Syn were 39% and 31% respectively (Figure 2B-C; I^2 = 78.08% and 94.83% respectively). CD56 was only assessed in one study which had a prevalence of 9% (data not shown)[33]. Combined analyses of two or more neuroendocrine markers had a higher pooled prevalence (51 %) than analyses of the single markers, however these studies also displayed considerable heterogeneity (Figure 2D; I^2 = 92.79%). Overall, the variation in reporting of neuroendocrine staining is consistent regardless of which of the three most common neuroendocrine markers were assessed.

Subgroup analyses and meta-regression reveals the importance of objective criteria to report neuroendocrine staining

Analyses to assess publication bias in studies using CgA showed no small study effects or publication bias (Figure S3). To examine the heterogeneity in prevalence of neuroendocrine marker staining and determine the influence on the findings, we performed a subgroup and meta-regression analysis of CgA studies for study characteristics (tissue type, criteria for neuroendocrine staining, antibody type, sample size), study design and study quality. We found the prevalence of neuroendocrine staining was significantly influenced by the reporting criteria used to classify a tumour as positive for neuroendocrine staining in both the sub-group analyses ($p<0.01$) and meta-regression ($p<0.001$; Figure 3A-B).

Prevalence was lowered to 26% in studies that used an objective criterion to report neuroendocrine staining (Figure 3A), when compared to studies using subjective criteria (44%) and studies that used any positive staining (55%: Figure 3A). Subgroup analysis on studies that used objective reporting criteria identified a significant difference between tissue types (Figure 3C; Figure S4C), where biopsy tissue was associated with significantly lower prevalence than in prostatectomy samples (11% vs 21%, p=0.02; Figure 3C). Subgroup analyses on studies that used any positive cells or subjective criteria, found differences in prevalence according to study design and antibody respectively (Figure S4A-B). Amongst the studies using objective criteria, there were varying threshold applied including clusters of

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cells, mean cells per field (>30), and percentage of positive tumour cells (>1%, 10%). A comparison of studies grouped by their specific objective criteria showed no significant difference in prevalence (p=0.33, Figure 3D).

Neuroendocrine staining does not correlate to tumour grade

To test the assumption that neuroendocrine staining correlates with higher Gleason score, we examined the prevalence of CgA staining in patient tumours with Gleason score ≤7 and Gleason score ≥8. When assessing studies using objective criteria there was no correlation between the prevalence of CgA staining and Gleason score (Figure 4). A similar correlation was observed in studies that used any positive staining (Figure S5). Thus, positive neuroendocrine staining was independent of tumour grade.

Objective reporting of neuroendocrine staining is prognostic

Analysis of the association between CgA staining and clinical outcomes found that tumours reported as positive for CgA staining using objective criteria were associated with accelerated biochemical recurrence (HR: 1.98 (95%CI = 1.49 to 2.65)) and poorer prostate cancer-specific survival (HR: 7.03 (95%CI = 2.55 to 19.39)), compared to patients reported negative for neuroendocrine staining (Figure 5A,D, Figure S6 for RR). There was no correlation for patients with neuroendocrine staining and clinical progression or overall survival (Figure 5B-C, Figure S6). Furthermore, the HRs for biochemical recurrence and prostate cancer-specific survival were significantly higher in studies using objective criteria compared to studies using any positive staining as the reporting criteria (Figure 5). Whilst multiple papers examined outcomes in tumours that were Gleason scores ≤ 7 [31, 72] (Table S2), the only study to define outcomes in patient with low risk tumours (Gleason score ≤ 6) using objective criteria was Weinstein et al. [78], who showed a correlation with accelerated biochemical recurrence (RR: 4.90 (95%CI = 2.21 to 10.9)): Figure S6).

Discussion

There is significant variation in the proportion of adenocarcinomas reported to have neuroendocrine cells and the prognostic significance of these lesions is unclear [3, 81]. This study demonstrated that the variation in prevalence is largely driven by the inconsistent criteria to classify a tumour as positive for neuroendocrine staining, and using objective criteria can reduce variation in reported prevalence, independent of Gleason score. Furthermore, when objective criteria are applied, the presence of neuroendocrine cells

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correlates with poor prognosis, even for low risk cancers. Together, this suggests that detection of neuroendocrine cells at diagnosis may be prognostic if objective criteria are applied, and that the clinical utility of reporting neuroendocrine staining in adenocarcinomas should be revisited.

While our analysis demonstrated the importance of objectively reporting neuroendocrine staining, the sample size was insufficient to be able to determine the exact threshold of neuroendocrine cells that should be applied. While the criteria in our included studies suggested that the threshold of prognostically significant cells will be small, i.e. >1% as reported by Krauss et al. [58], it will be essential for future studies to determine the exact proportion of neuroendocrine staining that is prognostic of increased risk of progression or death. Determining quantitative thresholds has been integral for inclusion of biomarker staining in other tumours, such as HER2 staining in breast cancer to identify patients who would benefit from chemotherapy and trastuzumab [82]. Additionally, our analysis showed that CgA is the most common neuroendocrine marker used and the evidence indicates that pathological assessment may only require a single marker for a reliable diagnosis. Future studies in prostate cancer will need to confirm a consistent approach for reporting neuroendocrine staining to identify patients at risk of poor prognosis. This will require determining clinically significant cut-offs for reporting neuroendocrine staining, whether cut-offs will need to be different for radical prostatectomy versus biopsy specimens, and evaluating the prognostic utility of other common neuroendocrine biomarkers such as synaptophysin.

Despite the finding that inconsistent reporting criteria significantly influenced the variation in neuroendocrine cell prevalence, a high degree of heterogeneity remained in subgroup analyses when the reporting criteria was taken into account. Several factors may have driven the heterogeneity across studies, including the use of different antibodies for staining, the approach for quantifying staining in each specimen, as well as inter-observer variation when assessing different thresholds. Together, this highlights the complexities of reporting prognostic biomarkers that require quantifiable thresholds. Therefore, if future studies confirm a prognostic utility of neuroendocrine biomarker staining, there will need to be a consensus amongst pathologists for the best approach to report staining. To achieve this, accurate methods of quantitation that can overcome inter-observer variation may be beneficial, such as artificial intelligence algorithms under development for prostate diagnosis.

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An unexpected finding of this study was that the presence of neuroendocrine staining reported with objected criteria was not different between Gleason score ≤7 and Gleason score ≥8 tumours, suggesting that neuroendocrine staining will be a prognostic indicator of poor outcomes independent of tumour grade. However, out analysis was limited by the fact that the correlation could not be performed on ISUP tumour grade groups, as the majority of studies were performed prior to implementation of the ISUP grade group system, and thus Gleason score 7 specimens would include both Grade group 2 and 3 tumours [83]. Future studies should use ISUP Grade group to determine the prevalence of neuroendocrine staining, as well as other clinical parameters such as tumour stage, to confirm if reporting of neuroendocrine differentiation is an independent prognostic variable of poor outcomes. Additionally, we identified that the correlation to poor patient outcomes was identified in low Gleason score cancers using objective criteria, although this was based on a single study which performed the analysis in prostatectomy specimens [78]. However, this is a critical finding and requires further validation in active surveillance cohorts, but if proven, indicates a strong rationale for pathologists to routinely stain for neuroendocrine markers during biopsy analysis to avoid offering active surveillance for patients who are not suitable.

Finally, these studies did not address the mechanism(s) of why patients with clinically significant neuroendocrine differentiation had a poor prognosis. A possible explanation is that these lesions of neuroendocrine cells are a precursor to aggressive neuroendocrine tumours that are detected later in disease progression. This was not assessed in the included studies as most were performed prior to 2015, when the clinical significance of neuroendocrine tumours in CRPC was not widely appreciated. While recent preclinical studies have demonstrated a relationship of neuroendocrine differentiation in adenocarcinomas to aggressive androgen independent tumours [84], this has not yet been proven in patients. Future studies should look at additional outcomes in patients to assess a relationship to aggressive neuroendocrine tumours such as clinical progression in the absence of PSA elevation. If a relationship is found, this would suggest that patients with clinically significant proportions of neuroendocrine cells require alternative monitoring and treatment options to PSA testing and hormone therapy.

The prognostic significance of neuroendocrine differentiation in prostate cancer has been controversial. This systematic review provides important insight into why there has been

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confusion in the literature, due to the inconsistent methods used over the past decades to assess positive staining for neuroendocrine markers. This study demonstrates the importance of defined reporting criteria when determining the prevalence of neuroendocrine staining in prostate adenocarcinomas. Importantly, we showed that objective reporting of neuroendocrine staining could identify clinically significant neuroendocrine cells that correlate with poor outcomes for the patient. Whilst the exact quantifiable thresholds are not yet defined, our findings provide a strong rationale to revisit the utility of performing neuroendocrine marker immunohistochemistry at prostate cancer diagnosis to stratify higher risk patients that require alternative clinical management.

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Role of the funder: The funders had no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; and the decision to submit the manuscript for publication.

Author Contributions

AK, RT, GPR and RAT had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: RT, GPR, RAT, SE

Acquisition of data: AK, RT

Analysis and interpretation of data: AK, RT, DI, NK,

Drafting of manuscript: AK, RT, RAT, GPR

Critical revision of the manuscript for important intellectual content: AK, MF, DC, DI, NK, SE, RT, GPR, RAT

Statistical analysis: AT, RT, DI, NK

Obtaining funding: RT, RAT, GPR

Supervision: SE, RT, GPR, RAT

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Table 1 - Characteristics of included studies

Author (year) & country	Study design	n	Tissue type	Tumour Grade	Neuroendocrine markers	Criteria for reporting neuroendocrine staining	REMARK score
Abrahamsson (1989) Sweden	Prospective cohort	25	TURP	Varying Gleason scores	CgA	Any positive cells	8/13
Adolf (2007) Denmark	Prospective cohort	77*	RP, TURP, M	Varying Gleason scores	CgA, NSE, Syn^{s}	≥ 1% cells	8/19
Ahlgren (2000) Sweden	Cross-sectional	53	RP	Varying Gleason scores	CgA	Cluster of positive cells	7/13
Almeida (2007) Brazil	Descriptive	40	RP	Gleason scores 7-9	CgA, Syn^{s}	Light expression	6/13
Angelsen (1997) Norway	Cross-sectional	12	TURP	Not stated	CgA, NSE^{s+c}	Any positive cells	8/13
Angulo (2016) Spain	Retrospective cohort	45	RP, TURP	Varying Gleason scores	CgA	Cluster of positive cells	10/19
Aprikian (1993) USA	Cross-sectional	31	RP	Varying Gleason scores	CgA, NSE^{s+c}	Any positive cells	8.5/13
Aprikian (1994) USA	Prospective cohort	50	M$^{B + LN}$	Not stated	CgA	Any positive cells	9/19
Augustin (2003) Germany	Cross-sectional study	48	RP	Gleason scores ≤6 & ≥9	CgA	≥ 1% cells	10/13
Autorino (2005) Italy	Prospective cohort	40	RP	Varying Gleason scores	CgA	> 10% cells	9/19
Berner (1993) Norway	Prospective cohort	47	TURP, B	Varying Gleason scores	NSE	Not defined	7.5/13
Berner (1995) Norway	Descriptive	80	TURP	Not stated	NSE	Not defined	8.5/13
Berruti (2010) Italy	Prospective cohort	414	B	Varying Gleason scores	CgA	Any positive cells	15.5/19
Bery (2020) France	Prospective cohort	314*	RP^{TMA}	Varying Gleason scores	CgA, Syn^{s}	Not defined	10/19
Birtle (2005) UK	Descriptive	33	TURP, B	Varying Gleason scores	CgA, CD56^{s}	≥ 10 cells	7.5/13
Bonkhoff (1993) Germany	Cross-sectional	11	RP	Not stated	CgA	Few scattered cells	6.5/13
Bostwick (1994) USA	Descriptive	26	RP	Varying Gleason scores	CgA, NSE^{s}	Not defined	10/13
Bostwick (2002) USA	Prospective cohort	196	RP	Varying Gleason scores	CgA	Any positive cells	13/19
Bozdogan (2005) Turkey	Cross-sectional	28	RP, TURP, B	Varying Gleason scores	CgA	Not defined	8.5/13
Bubendorf (1996) Switzerland	Prospective cohort	137	RP	Varying Gleason scores	CgA, NSE^{c}	Any positive cells	9.5/19
Casella (1998) Switzerland	Prospective cohort	105	B	Varying Gleason scores	CgA	Any positive cells	9/19
Chen (2020) China	Retrospective cohort	630	B	Varying Gleason scores	CgA, Syn^{c}	> 5% cells	13/19

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Cindolo (2007) Italy	Cross-sectional	116	RP, TURP, B	Varying Gleason scores	CgA	≥ 5% cells	7.5/13
Cindolo (2011) Italy	Prospective cohort	628	RP+TURP^{TMA}	Varying Gleason scores	CgA	≥ 5% cells	11.5/19
Cohen (1994) USA	Prospective cohort	38	RP	Varying Gleason scores	CgA, NSE^{s+c}	Any positive cells	10.5/19
Cohen (1991) South Africa	Prospective cohort	90	TURP, B	Varying Gleason scores	CgA, NSE^{c}	Any positive cells	9/19
Cohen (1995) NZ	Cross-sectional	52	RP, TURP	Varying Gleason scores	CgA, NSE^{c}	Any positive cells	6.5/13
Genitsch (2017) Switzerland	Prospective cohort	119	RP^{TMA}	Varying Gleason scores	CgA	Any positive cells	13/19
Grimaldi (2006) Italy	Descriptive	26	RP	Not stated	CgA	> 10% cells	7.5/13
Grobholz (2005) Germany	Prospective cohort	73	RP	Varying Gleason scores	CgA	Cluster of positive cells	11.5/19
Gunia (2008) Germany	Prospective cohort	528	RP	Varying Gleason scores	CgA	Cluster of positive cells	15/19
Heinrich (2011) Germany	Prospective cohort	175	RP	Varying Gleason scores	CgA	Mean ≥ 30 cells per field	8/19
Hirano (2004) Japan	Cross-sectional	38	RP	Varying Gleason scores	CgA	Any positive cells	9/13
Ishida (2009) Japan	Prospective cohort	50	RP	Varying Gleason scores	CgA	≥ 10 cells	8/19
Ito (2001) Japan	Cross-sectional	44	RP	Varying Gleason scores	CgA	Not defined	6/13
Jeetle (2012) UK	Prospective cohort	806	TURP^{TMA}	Varying Gleason scores	CgA	Any positive cells	14/19
Kaur (2020) USA	Prospective cohort	267	RP^{TMA}	Varying Gleason scores	CgA	≥ 5% cells	12.5/19
Köllermann (2001) Germany	Cross-sectional	20	RP	Not stated	CgA	Any positive cells	5.5/13
Komiya (2013) Japan	Prospective cohort	20	B	Average Gleason score 8	CgA, NSE^{c}	≥ 50% cells	8.5/13
Krauss (2014) USA	Prospective cohort	289	B	Gleason scores 7-10	CgA	> 1% cells	13.5/19
Krijnen (1997) Netherlands	Prospective cohort	72	TURP	Varying Gleason scores	CgA	Any positive cells	14.5/19
Krupski (2000) USA	Prospective cohort	71	RP	Varying Gleason scores	CgA	Any positive cells	10.5/19
Ma (2010) Japan	Prospective cohort	114	RP	Not stated	CgA	Mean ≥ 30 cells per field	14/19
Marcu (2010) Romania	Descriptive	43	B	Varying Gleason scores	CgA, NSE^{s}	Any positive cells^{CgA} ≥ 33% positive cells^{NSE}	6.5/13
McWilliam (1997) UK	Prospective cohort	92	TURP	Varying Gleason scores	CgA, NSE^{s+c}	> 10% cells	10/19
Ohara (2008) Japan	Prospective cohort	98	RP	Varying Gleason scores	CgA	Any Positive cells	11/19

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Pascale (2016) Italy	Prospective cohort	166*	RP+TURP^{TMA}	Varying Gleason scores	CgA, NSE, Syn^{s}	Diffuse staining	12.5/19
Puccetti (2005) Italy	Descriptive	105	RP	Varying Gleason scores	CgA	> 1% cells	8/13
Quek (2006) USA	Prospective cohort	95	RP	Not stated	CgA	≥ 5% cells	10/13
Sainio (2018) Finland	Prospective cohort	89*	RP^{TMA}	Not stated	CgA, NSE, Syn^{s}	≥1% cells	10/19
Segawa (2001) Japan	Prospective cohort	42	RP, TURP, B	Varying Gleason scores	CgA, Syn^{c}	Any positive cells	11/19
Shimizu (2007) Japan	Cross-sectional	70	RP	Varying Gleason scores	CgA, Syn^{s}	Any positive cells	10/13
Tarjan (2010) Sweden	Descriptive	39	TURP, B	Varying Gleason scores	CgA	Single/groups of cells	9/13
Theodoropoulos (2005) Greece	Prospective cohort	81	RP, TURP	Varying Gleason scores	CgA, NSE^{s+c}	≥ 2% cells	12/19
Tokunaga (2005a) Japan	Cross-sectional	20	RP	Varying Gleason scores	CgA	Any positive cells	8.5/13
(2005b)		69	B	Varying Gleason scores	CgA	Any positive cells	
Van de Voorde (1995) Belgium	Descriptive	107	RP	Varying Gleason scores	CgA	Any positive cells	9.5/13
van Leenders (2001) Netherlands	Cross-sectional	38	M^{LN+B}	Not stated	CgA	Not defined	7.5/13
Veltri (2008) USA	Prospective cohort	105	RP	Varying Gleason scores	CgA	> 38 cells	12.5/19
Wafa (2007) Canada	Cross-sectional	84	RP^{TMA}	Varying Gleason scores	CgA	Any positive cells	9.5/13
Weinstein (1996) USA	Prospective cohort	104	RP	Varying Gleason scores	CgA	Any positive cells	8/19
Xiao (2020) USA	Cross-sectional	47	RP+TURP^{TMA}	Gleason scores 7-8	Syn	>10% cells	7.5/13
Xing (2001) USA	Prospective cohort	44	RP	Varying Gleason scores	CgA	Not defined	9.5/19

B = Biopsy, C = markers assessed in combination, CgA = Chromogranin A, M = Metastases, M^{B} = Bone metastases, M^{LN} = Nodal metastases resected via lymphadenectomy, NSE = Neuron specific enolase, RP = Radical prostatectomy, S = Markers assessed separately, Syn = Synaptophysin, TMA = Tissue microarray, TURP = Transurethral resection of prostate, * = Study had different sample sizes for each neuroendocrine marker

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Figure Legends

Figure 1 - PRISMA diagram for study selection. NE = neuroendocrine.

Figure 2 - Meta-analyses of neuroendocrine staining according to different neuroendocrine markers. Forest plots showing the prevalence of neuroendocrine staining according to immunohistochemistry for (**A**) chromogranin A (CgA), (**B**) neuron specific enolase (NSE), (**C**) synaptophysin (Syn), or (**D**) two or more neuroendocrine markers assessed in combination. Weight (%) represents the contribution of each study to the analyses proportionate to sample size. CI= confidence interval.

Figure 3 – Subgroup and meta-regression analyses of chromogranin A staining. (**A**) Subgroup and (**B**) multivariable meta-regression of the prevalence of chromogranin A (CgA) staining according to study characteristics, study design, study quality and sample size (meta-regression only). N represents the number of studies in each subgroup category. Subgroup plots of the prevalence of CgA staining reported using objective criteria in (**C**) prostatectomy and biopsy tissues and (**D**) prostatectomy tissue according to specific objective reporting criteria. Weight (%) represents the contribution of each study to the analyses proportionate to sample size. CI = confidence interval, NE = neuroendocrine.

Figure 4 - Correlation of objective reporting of chromogranin A staining to tumour grade. Subgroup plot of the prevalence of chromogranin A staining reported using objective criteria in tumours with Gleason score ≤7 and Gleason score ≥8. Weight (%) = represents the contribution of each study to the analyses proportional to sample size. CI= confidence interval.

Figure 5 - Hazard ratio of clinical outcomes in patients with chromogranin A staining subgrouped by reporting criteria. (**A-D**) Subgroup plots showing the risk (Hazard Ratio) of (**A**) biochemical recurrence, (**B**) clinical progression, (**C**) overall survival and (**D**) prostate cancer-specific survival in patients with chromogranin A staining reported using different criteria. CI= confidence interval, cpf= cells per field.

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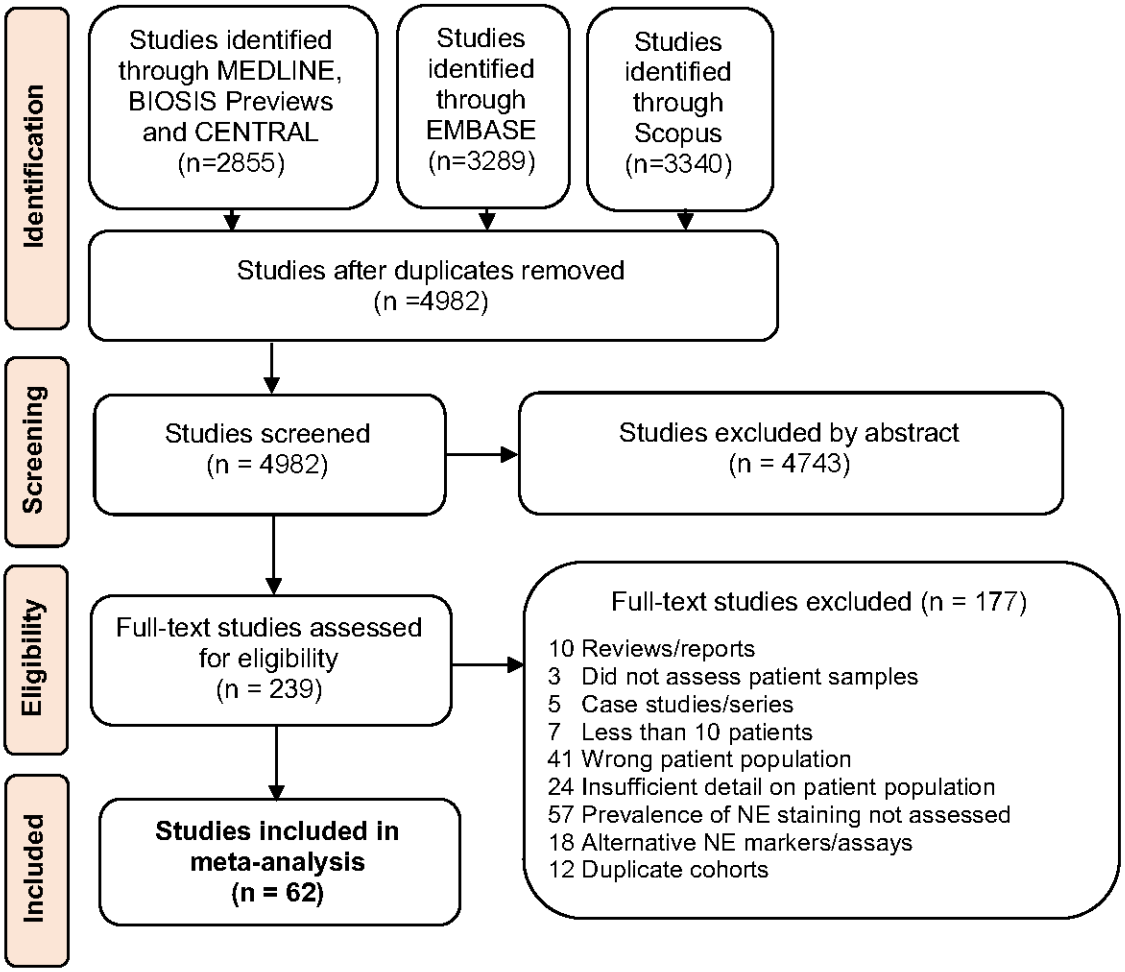

Figure 1

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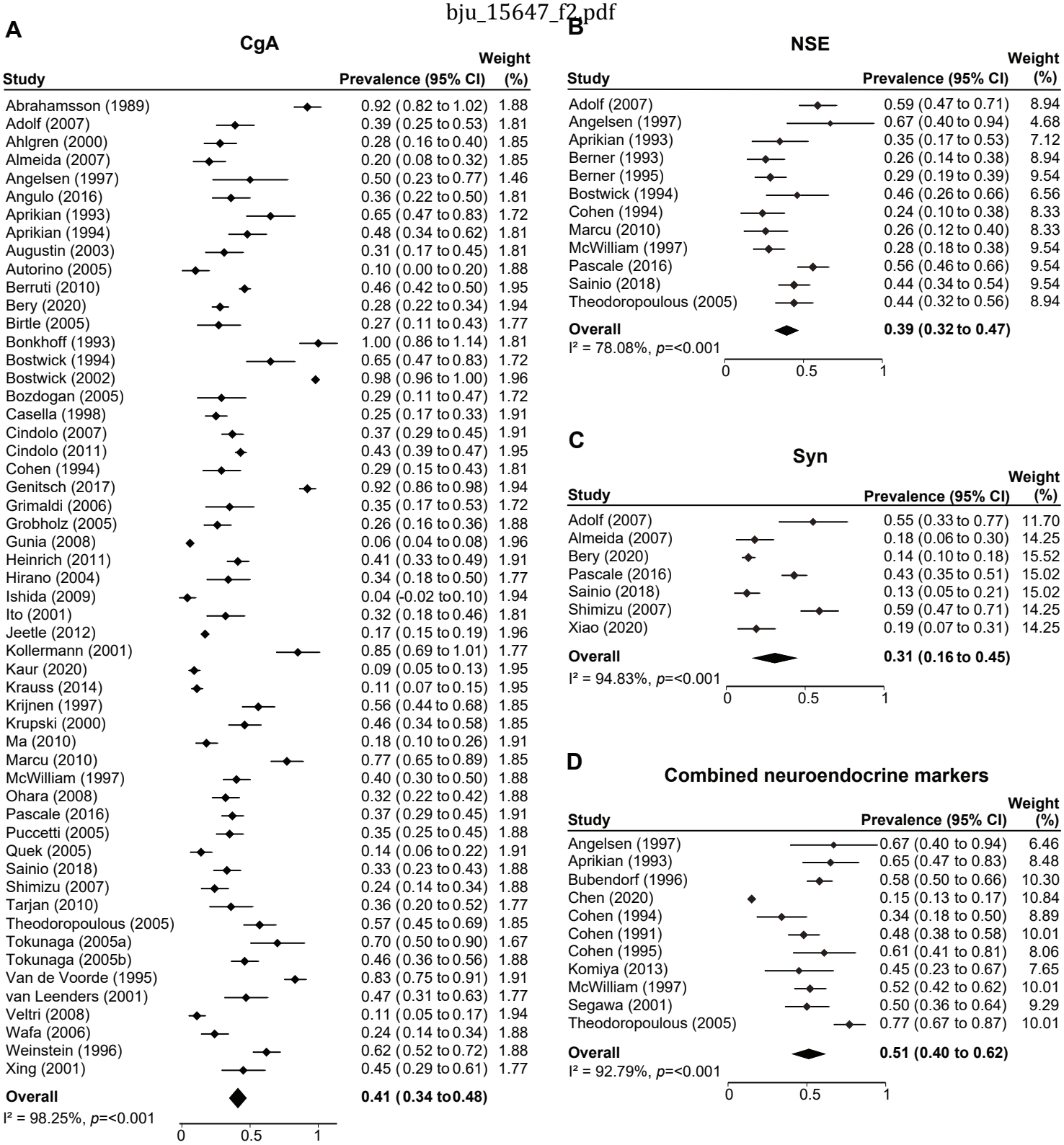

Figure 2

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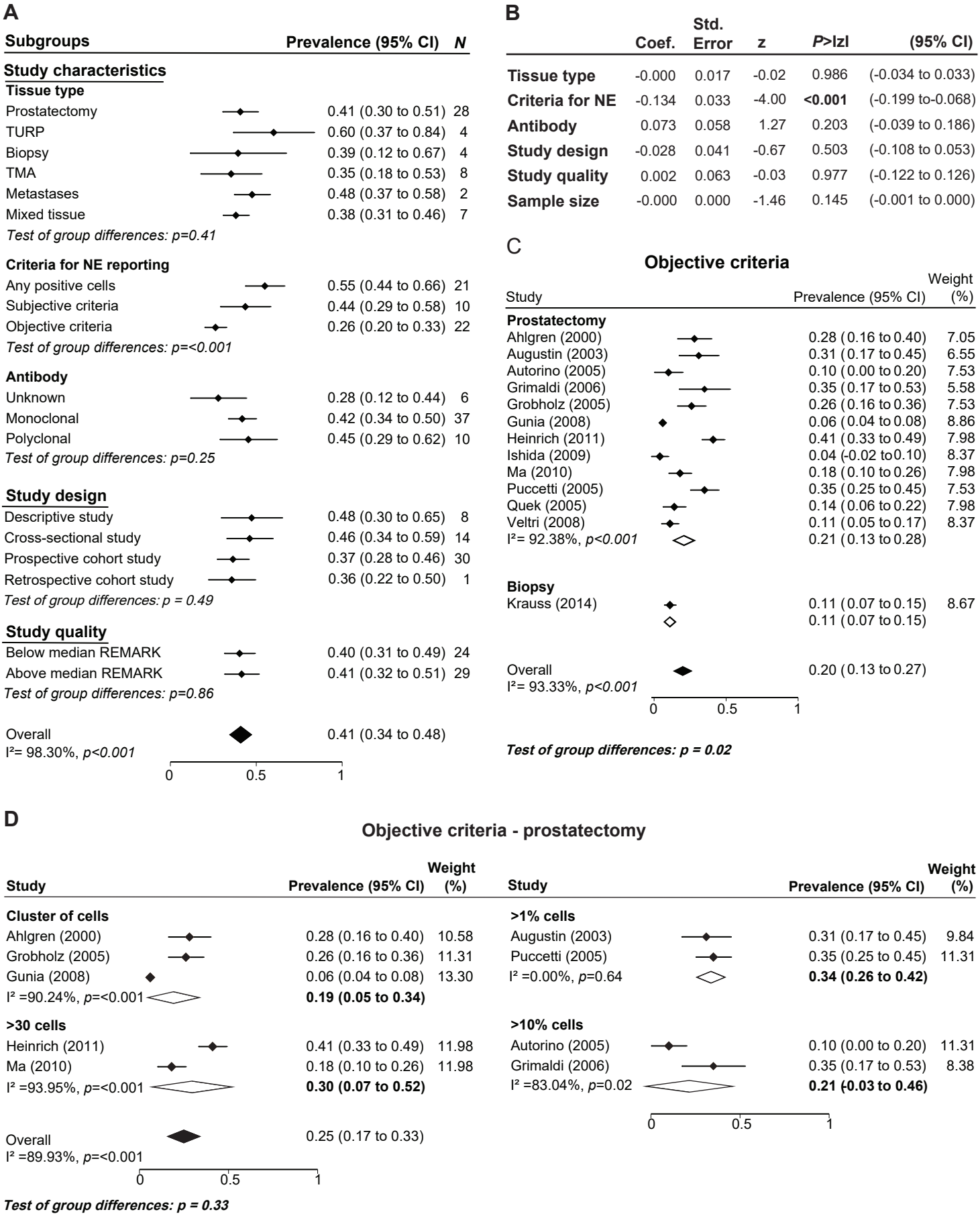

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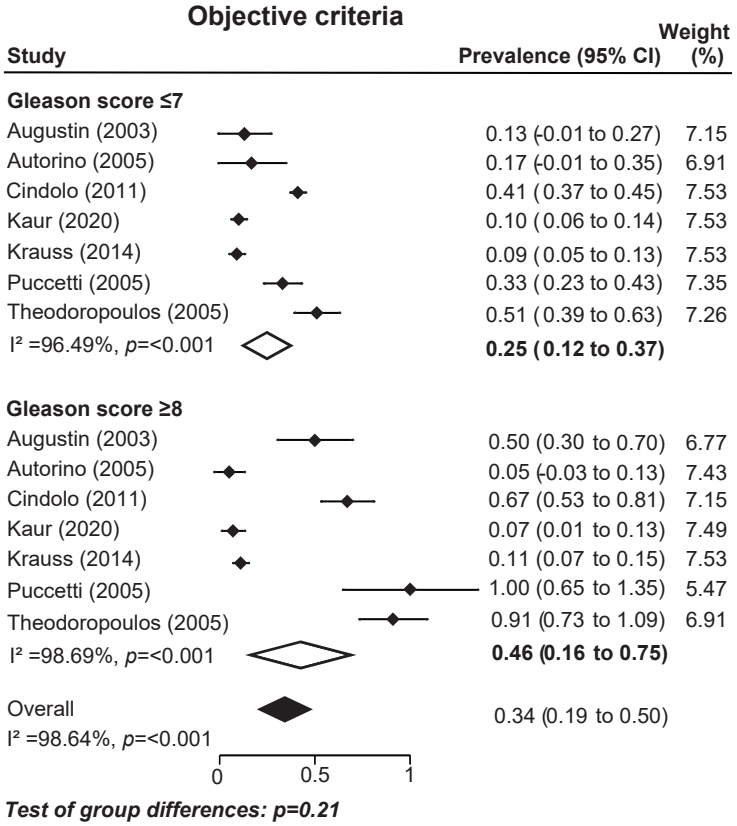

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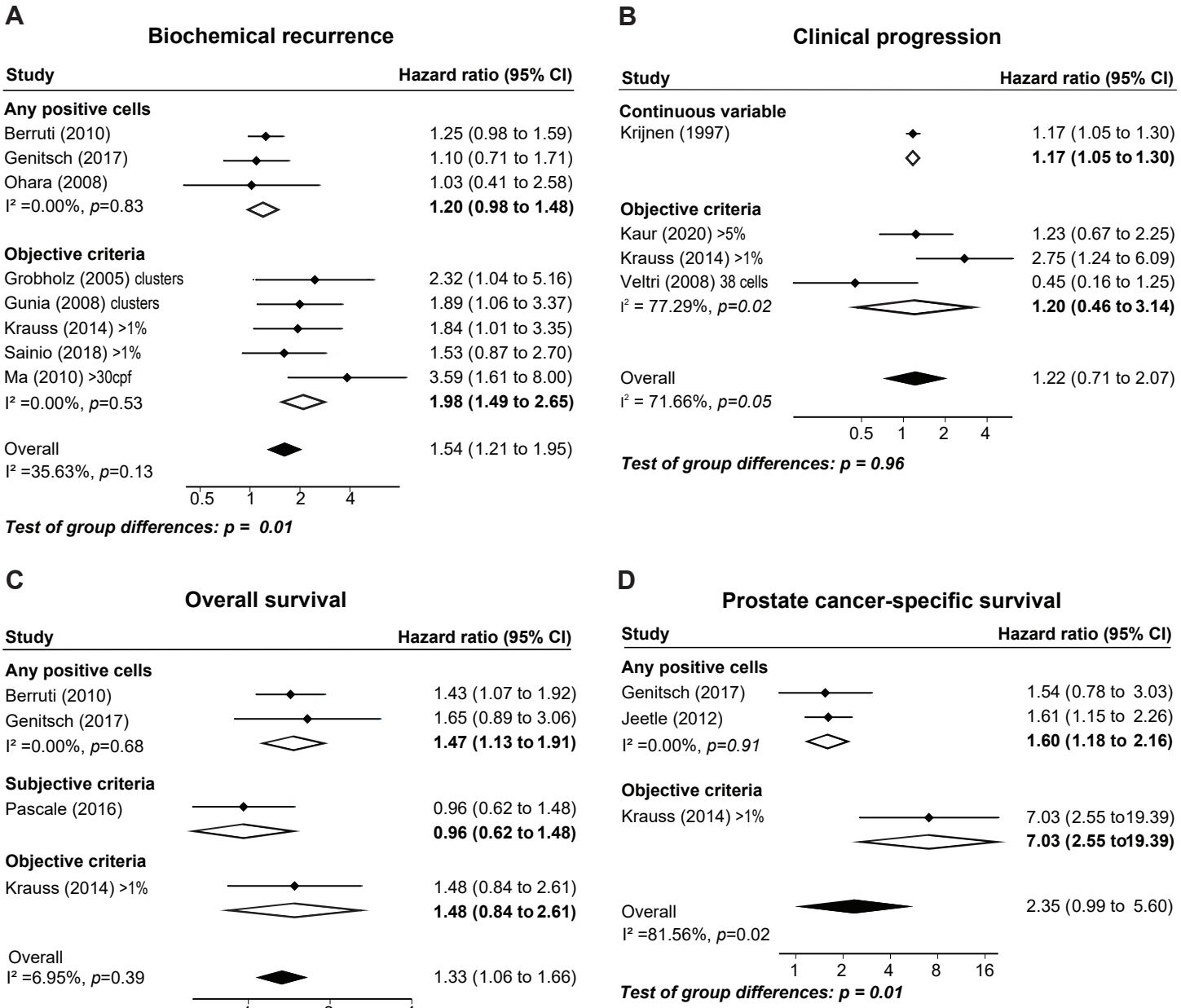

Figure 5

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