Diagnostic value of Telomerase Activity in patients with Bladder cancer: a meta-analysis of diagnostic test

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Abstract

Background: This study aimed to evaluate the diagnostic value of telomerase activity (TA) for bladder cancer (BC) by meta-analysis. Methods: We conducted a systematic search of studies published on PubMed, Embase, and Web of Science up to June 1, 2019. We used Stata 15 and Review Manager 5.3 for calculations and statistical analysis. Results: To evaluate the diagnostic value of TA for BC, we performed a meta-analysis on 22 studies, with a total of 2867 individuals, including sensitivity, specificity, positive and negative likelihood ratio (PLR, NLR), diagnostic odds ratio (DOR), and 95% confidence intervals (CIs). The pooled parameters were calculated from all studies and we found a sensitivity of 0.79 (95% CI: 0.72 – 0.84), a specificity of 0.91 (95% CI: 0.87–0.94), a PLR of 8.91 (95% CI: 5.91–13.43), an NLR of 0.24 (95% CI: 0.15–0.37), a DOR of 37.90 (95% CI: 23.32–61.59), and an AUC of 0.92 (95% CI: 0.90–0.94). We also conducted a subgroup analysis based on the different stages and grades of BC. Results from the subgroup analysis showed that there was no significant difference in TA in either high and low stages of BC, but that low-grade tumours had a lower TA than high-grade tumours. Conclusions: In BC, there is a high overall diagnostic value for TA, and this could provide an alternative to cystoscopy for staging and grading of tumours. The tumor characteristics also showed a good diagnostic value

Background

Bladder cancer (BC) is a malignant tumor with very high invasiveness and is one of the ten most common cancer types occurring in both males and females [1, 2]. BC can generally be identified using pain-free methods such as macroscopic haematuria or microscopic haematuria, but these methods usually lead to a poor prognosis [3].

Due to the lack of specific clinical symptoms in BC patients, early diagnosis has a great impact on treatment and prognosis [4]. Generally, urine cytology, histology, and cystoscopy are the most common methods for diagnosis of BC [5]. Cystoscopy is the gold standard diagnostic method for BC. Its intuitive characteristics are very reliable in the diagnosis of BC, but this invasive operation causes pain, and it is costly which affects its clinical frequency and subsequent follow-up [6]. The search for a better, lower-risk, accurate and easy-to-manage methodology for the diagnosis of BC has been ongoing [7].

Detection of telomerase activity（TA） is a non-invasive and effective auxiliary test for the diagnosis of BC [8]. Telomerase is correlated to the maintenance of the telomere length in tumor cells and the infinite division of cells. Telomerase activity is present in tumor cells but it is not typically detected in the normal tissues surrounding the tumor [9]. Compared with cystoscopy, the detection of TA can be usually performed using a urine or bladder irrigation solution, which greatly reduces the patient's fear of medical examination, and also facilitates follow-up [10].

Non-invasive diagnostic methods have become a popular and emerging field. There are many studies reporting the accuracy of TA in the diagnosis of BC. However, these diagnostic capabilities are reported by different research groups and thus have significant differences between them. Taking into account the

limitations of single studies, we performed a meta-analysis based on several research samples and used statistical calculations to better understand the diagnostic efficiency of TA in patients with BC. Studies have previously revealed the relationship between telomere length and various cancers [11]. Based on these studies, we further explored the relationship between TA and BC, with the objective of determining the status of telomeres and telomerase activity and their role in BC.

Methods

Literature search and eligibility criteria

We systematically retrieved relevant literature from the PubMed, Embase, and Web of Science databases from inception to June 1st, 2019. We used TA, BC, and urine as the search terms and the search language was limited to English. We also searched the relevant references' directories to avoid missing other relevant documents.

Studies that meet the following requirements were included in our research: patients diagnosed with BC using the gold standard cystoscopy, studies with the diagnostic value of TA reflected in the research article, and studies with sufficient data on true positive (TP), false positive (FP), false negative (FN), and true negative (TN). Duplicate articles, insufficient quality, studies focusing on other diseases, letters, comments, case reports, and editorials were excluded from our analysis. The review process was assessed by two authors, independently.

Data extraction

We included the following data from each study in our meta-analysis: first author, publication year, region, sample size, and four datasets (TP, FP, FN, TN). Two authors (PL and CDH) extracted information using a standard excel worksheet and verified the data, respectively. A third investigator (RZJ) resolved any disparities arising in the datasets.

Quality evaluation

We used the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) to assess the quality of the included studies. We also used a quantitative method to assess the selected studies. The QUADAS-2 included 14 items [12]. Each key domain included two sections, the risk of bias and applicability. We determined a low risk of bias when all signalling questions for a domain were answered 'yes'. If the answer to any of the questions was 'no', then potential bias was considered. Concerns about applicability were determined as 'low', 'high', or 'unclear'.

Statistical analysis

We used Stata 15 (StataCorp LP, University City, Texas, USA) and Review Manager 5.3 for the statistical analysis. Using a Q test and I^2 to evaluate the heterogeneity of the study, I^2>50% improvement was considered as significantly heterogeneous [13]. We used a bivariate model to calculate the pooled

sensitivity, specificity, positive and negative likelihood ratios (PLRs and NLRs), diagnostic odds ratio (DOR) and the 95% confidence interval (CI) [14]. We calculated the area under the receiver operator characteristic curve (SROC, AUC). AUC varied from 0.5 to 1. If the area was equal to 1, then diagnosis had perfect discrimination. If the area was 0.5, then diagnostic ability was considered as poor [15]. Deeks funnel plot was used to assess the publication bias, and Fagan plots showed the relationship between the prior probability, the likelihood ratio, and posterior test probability [16]. $P<0.05$ was considered to be statistically significant.

Results

Study selection and study characteristics

Figure 1 presents the literature search selection process. Initially, we identified a total of 515 studies in the selected databases. Of these, 195 duplicate records were excluded. After analysis of the title, abstract, and topic, 260 other articles were excluded. Sixty articles remained, which were subjected to full text analysis and assessment of eligibility, following which another 41 articles, unrelated to diagnostic value and 4 duplicates, were excluded. Similarly, 8 studies were excluded due to insufficient data. Finally, we included 22 studies in our qualitative and quantitative analysis [7, 9, 10, 17-35].

In Table 1 the characteristics that were included in the meta-analysis of TA for BC for the 22 selected articles are presented. These articles spanned from 1997 to 2010. A total of 2867 sample individuals worldwide were included in our analysis. Most of these were from multicentre studies. Sample sizes ranged from 42-185, 5 studies were conducted in Asia (Japan and Israel), 6 in the United States, 10 in Europe (UK, Germany, Italy and Poland), and one in Africa (Egypt). The four-fold table data is presented in Table 1.

Quality assessment

Table 1 lists the quality scores for each study. Each article scored 11 points or higher. According to the QUADAS-2 scoring standard, 18 studies were classified with a middle to high score.

Pooled diagnostic values

Since the value of I^2 was greater than 50%, the random effects model was used to combine sensitivity and specificity. The diagnostic value of TA for the detection of BC is shown in Table 2. The overall sensitivity and specificity were recorded as 0.79 (95% CI: 0.72–0.84) and 0.91 (95% CI: 0.87–0.94, Figure 2), respectively. The Youden Index was 0.7. The pooled PLR was 8.91 (95% CI: 5.91–13.43), NLR was 0.24 (95% CI: 0.15–0.37) and DOR was 37.90 (95% CI: 23.32–61.59). The overall SROC curve is shown in Figure 3, with an AUC of 0.92 (95% CI: 0.90–0.94). The Fagan plot is shown in Figure 4. The prior probability was 20%, and the post-test probability was 69% for LR-positive and 6% for LR-negative. The diagnostic accuracy for detecting TA in BC was found to be generally better.

Subgroup analyses

We performed a subgroup analysis of TA based on different stages and grades of the tumours. We specified Tis, Ta, or T1 for a low stage tumor, and T2 and above for a high stage tumor. Similarly, we specified that grade 1 is a low-grade tumor and grade 2-3 is a high-grade tumor. According to the results of the heterogeneity test, we used a fixed model for meta-analysis for both grade and stage, and the results and forest map are shown in Figure 5. In our comparison of the different subgroups, the P value was >0.05, suggesting that there was no significant difference in TA in either the high or low stages of a tumor（Figure 5）. When comparing the different grades, we observed a P = 0.001, suggesting that low-grade tumours have lower TA than high-grade tumours.

Publication bias

The Deeks plot showed there was no publication bias (P=0.83, Figure 6).

Discussion

To our knowledge, this is the first meta-analysis of the diagnostic efficacy for TA in BC. We found that TA is optimal among various other indicators and proved to be an excellent diagnostic.

BC, as a malignant tumor with high morbidity and mortality, has received wide attention, both for its diagnosis and treatment [36]. As accepted, cystoscopy has been the gold standard for the diagnosis of BC. Despite its reliability, as an invasive examination, it is performed under local anaesthesia, causing strong discomfort to patients [7]. A simpler diagnostic would be preferable and would also minimize the damage caused by the examination. There is a shift in the continuous detection and development of BC test methods from macro to micro, and role of markers in urine is being explored for the detection and diagnosis of BC [18, 19].

Telomeres are composed of repeated gene sequences and related proteins. Their main role is to avoid end-to-end fusion and nuclear cleavage during chromosome division [18]. Telomerase reverse telomeres shortening during cell division. This is one of the essential processes for the permanent life of tumor cells [9]. We hypothesized that in tumor cells, telomerase activity would be higher than in normal cells. Many scholars have studied the relationship between TA and BC, but due to limitations in detection technology and in sample size, the conclusions were so far inconsistent. We integrated and analysed research done by other authors and included a sample group large enough for performance of meta-analysis, aiming at comprehensive evaluation of the diagnostic validity for looking into TA in BC, with a goal of providing better guidance for clinical practice.

A number of studies have shown that the sensitivity of the telomerase assay for urothelial carcinoma is lower in voided urine specimens than in bladder washings [17, 22, 24]. However, urine is easier to obtain than bladder washings and also easier to collect from the patient’s perspective. In our meta-analysis, the overall sensitivity was 0.79 (95% CI: 0.72–0.84), the specificity was 0.91 (95% CI: 0.87–0.94) and the

Youden index was 0.7. AUC was 0.92 (95% CI: 0.90–0.94), which was in line with our initial predictions. Using these composite indicators, we showed that TA could be a good and accurate indicator for the diagnosis of BC. A diagnostic test can typically be considered to have a high value when both sensitivity and specificity are >0.7. In this study, consistently with our predictions, the sensitivity results reached this value for 16 articles, again indicating the superiority of TA in the diagnosis of BC. However, the sensitivity values provided in the other 2 studies were significantly lower [17, 22]. The reason for this analysis was that, due to technical level of the test, the sample size, and bias between the samples, it might have lead to different final results. In relation to the specificity results, 21 of the included studies reached 0.7 or higher, showing that the results were not significantly different between the studies and confirming our hypothesis and indicating the excellent specificity of TA for the diagnosis of BC. The higher the value of DOR, the better the diagnostic ability for the selected method. In our study, the DOR value was 37.90 (95% CI: 23.32–61.59), suggesting that the overall accuracy was high. The overall PLR value was 8.91 (95% CI: 5.91–13.43), suggesting that patients with BC have a TA 8.91 times higher than normal, and a total NLR of 0.24 (95% CI: 0.15–0.37), meaning that normal individuals suffering from BC was of 25%. In the judging criteria, PLR>10, NLR<0.1, the diagnostic efficiency for this method was higher. Taking this aspect into account, we can conclude that the diagnostic efficiency of TA for BC is suboptimal.

To investigate the TA relationship between different staging and grading, we performed a subgroup analysis. In terms of staging, we considered Tis, Ta, and T0 as low-stage tumors, while T2-T4 as high stage. When grading, grade 1 was considered a low-grade tumor, and grades 2 and 3 were considered high-grade tumours. Thus, through meta-analysis, the association between these was evaluated. We found there was no absolute difference in TA between high-stage and low-stage tumours (P>0.05) or between different grades, using meta-analysis. Our results showed that the TA in low-grade tumours was significantly lower than in high-grade tumours (P=0.001). We believe that this is because the higher the grade, the lower the degree of differentiation, the stronger the invasive ability, and the higher the TA, consistently with results reported by Bravaccini *et al.* [7]. Detecting TA is not the only non-invasive method used in the diagnosis of BC, and other markers such as nuclear matrix protein (NMP)-22, bladder tumor antigen (BTA), Cytokeratin 20 could also be evaluated. Studies have reported that BTA and cytokeratin 20 are not sensitive markers for low-grade tumours. For grade 1 tumours, the sensitivity of BTA and cytokeratin were 13% and 6%, respectively, and NMP-22 had a specificity of 70% in the diagnosis of BC [9, 22, 25]. Therefore, as individual indicators, these markers may work better than invasive methods, but the diagnostic performance should take into consideration composite indicators. As seen by our meta-analysis, the overall diagnostic ability of TA proved to be superior.

We followed the PRISM guidelines for our meta-analysis [37]. However, at present, our meta-analysis still has some limitations. Firstly, of all the studies included here, most of the research samples were from Europe and the United States, which may skew our research due to racial differences. Secondly, in each group of controlled studies, the patients studied may have presented with other diseases. Since the mechanisms are unknown for these, the interactions between the different diseases may have lead to changes in the accuracy of our results.. Due to the influence of the original data, we were unable have sufficient detail on the different stages and grades of tumours. Compared with cystoscopy, although not

currently applied in the clinic, TA does have a higher advantage in the diagnosis of BC due to its relatively high sensitivity and non-invasiveness. A larger sample size, as well as a more rigorous study design, and longer follow-up randomized controlled trials will be needed to validate our results in the future.

Conclusions

Overall, our study demonstrates that TA has a high overall diagnostic value and demonstrates superiority when compared to other methodologies for BC diagnosis. Therefore it is expected to provide an alternative to cystoscopy for staging and grading of tumours. The tumor characteristics also have a good diagnostic value. In future, we would like to further our research in order to confirm our findings.

Abbreviations

BC, Bladder Cancer TA, Telomerase Activity QUADAS-2, Quality Assessment of Diagnostic Accuracy Studies 2; TP, true positives; FP, false positives; FN, false negatives; TN, true negatives; ROC, Receiver Operating Characteristic; SROC, summary receiver operator characteristic; PLRs, positive likelihood ratios; NLRs, negative likelihood ratios; DORs, diagnostic odds ratios; CI, confidence intervals.

Declarations

Ethics approval and consent to participate

Not applicable. There are no details on individuals reported within this manuscript, and no consent for publication was collected.

Consent to publication

Not applicable.

Availability of data and materials

All data generated and analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

Conceived and designed the experiments: PL. Analysed the data: LJZ and RZJ.

Contributed reagents/materials/analysis: PL, CDH and LJZ. Wrote the manuscript:

PL, LJZ, and CDH. All authors have read and approved the final manuscript.

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Figures

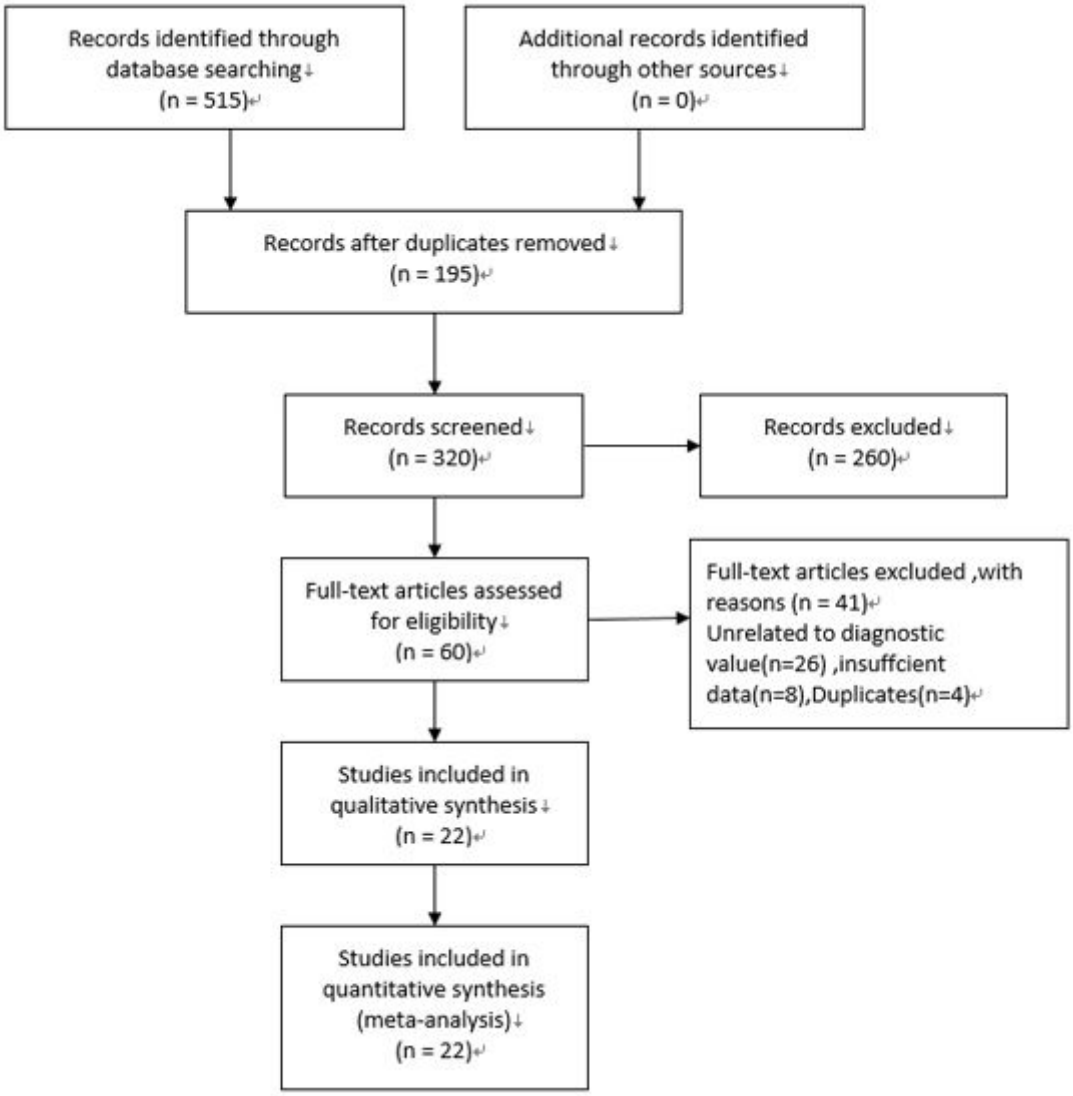

Figure 1

Flow diagram depicting the selection process for all articles found in literature.

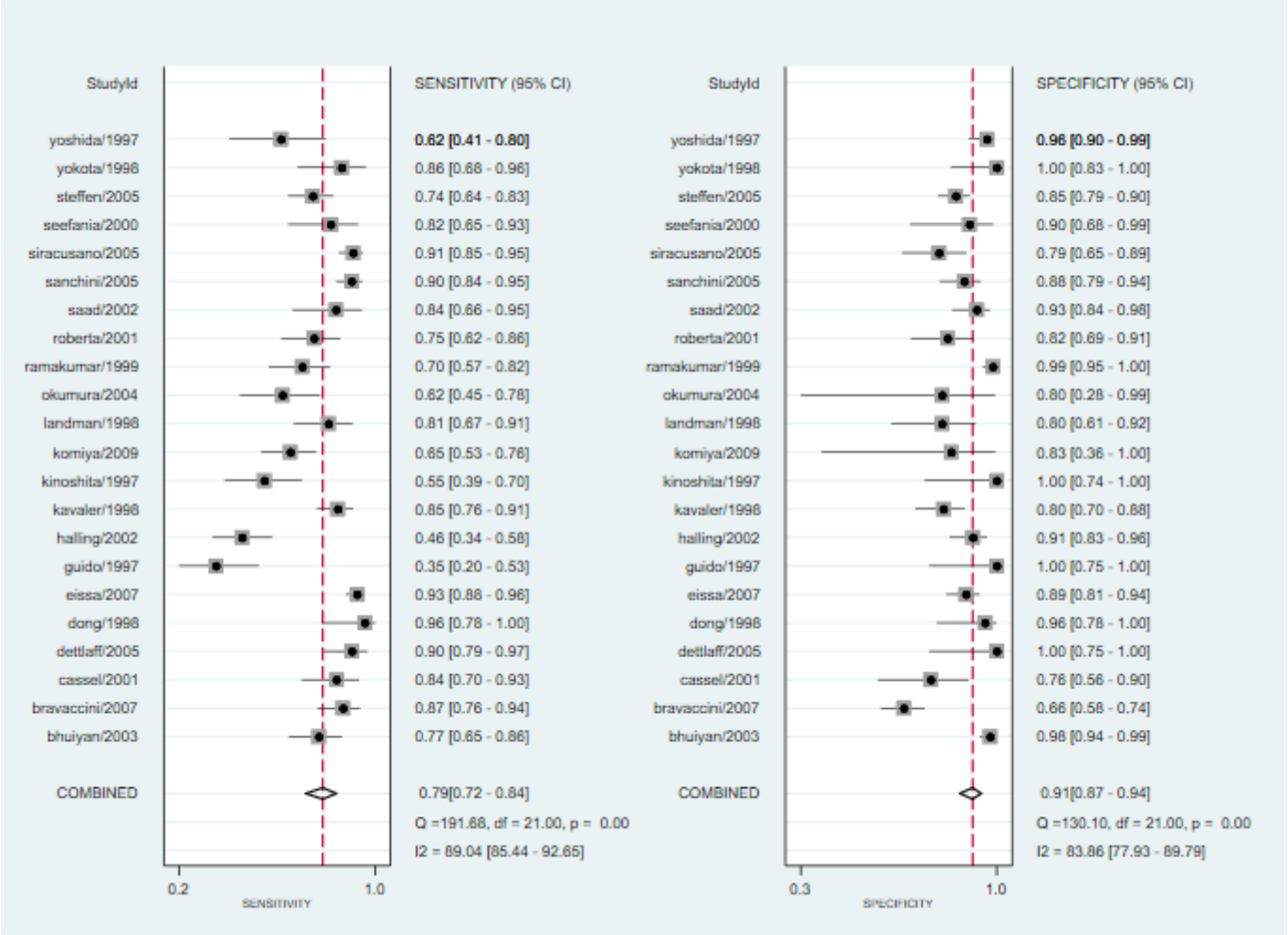

Figure 2

Forest plot of pooled sensitivity and specificity of Telomerase Activity for Bladder Cancer.

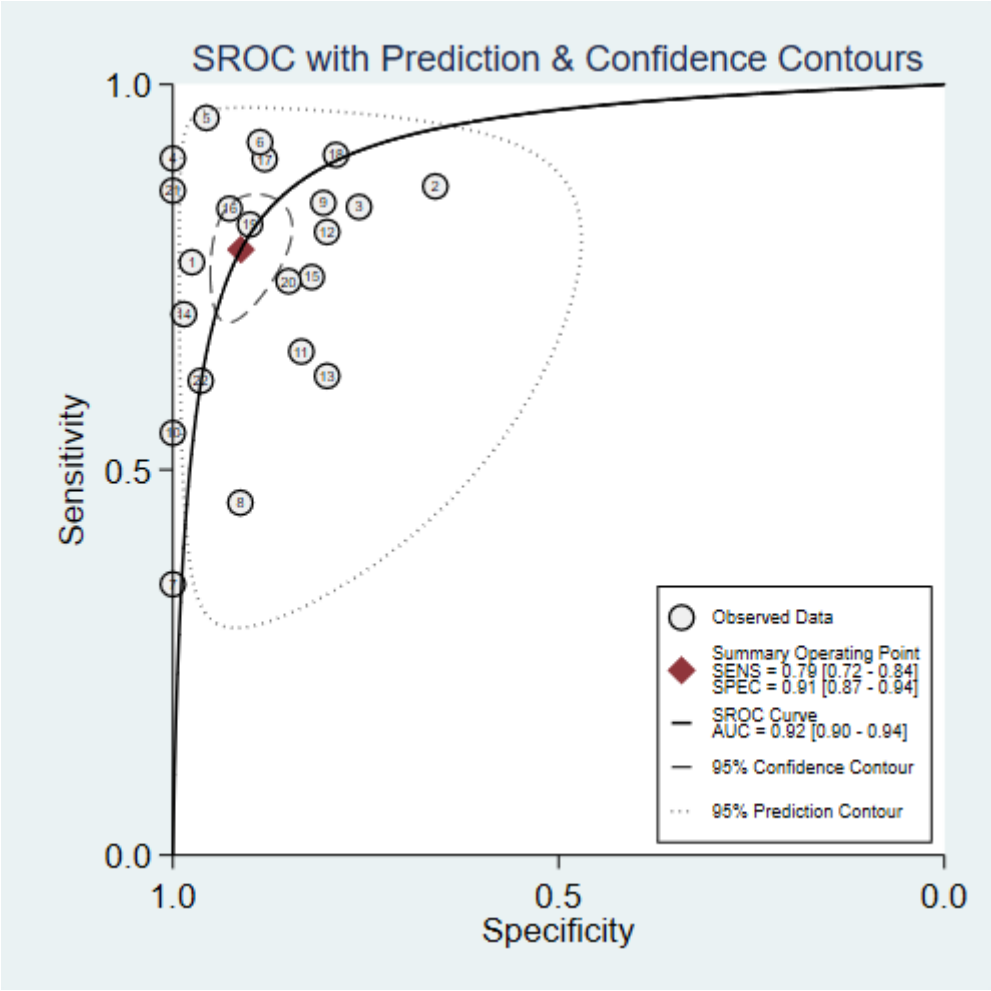

Figure 3

The SROC curve of Telomerase Activity for Bladder Cancer.

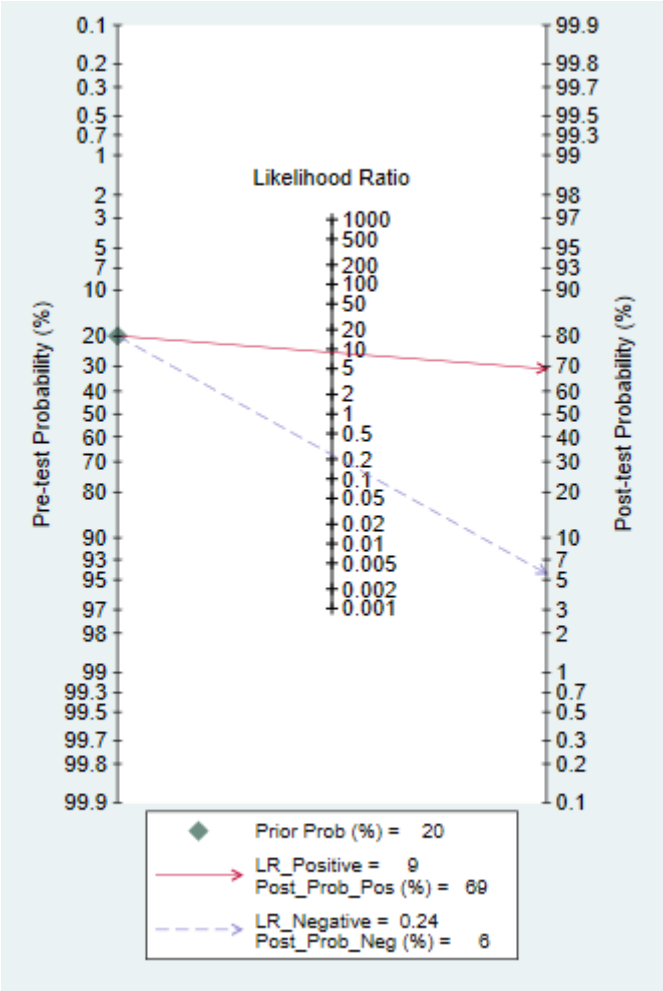

Figure 4

Fagan diagram evaluating the overall diagnostic value of Telomerase Activity for Bladder Cancer.

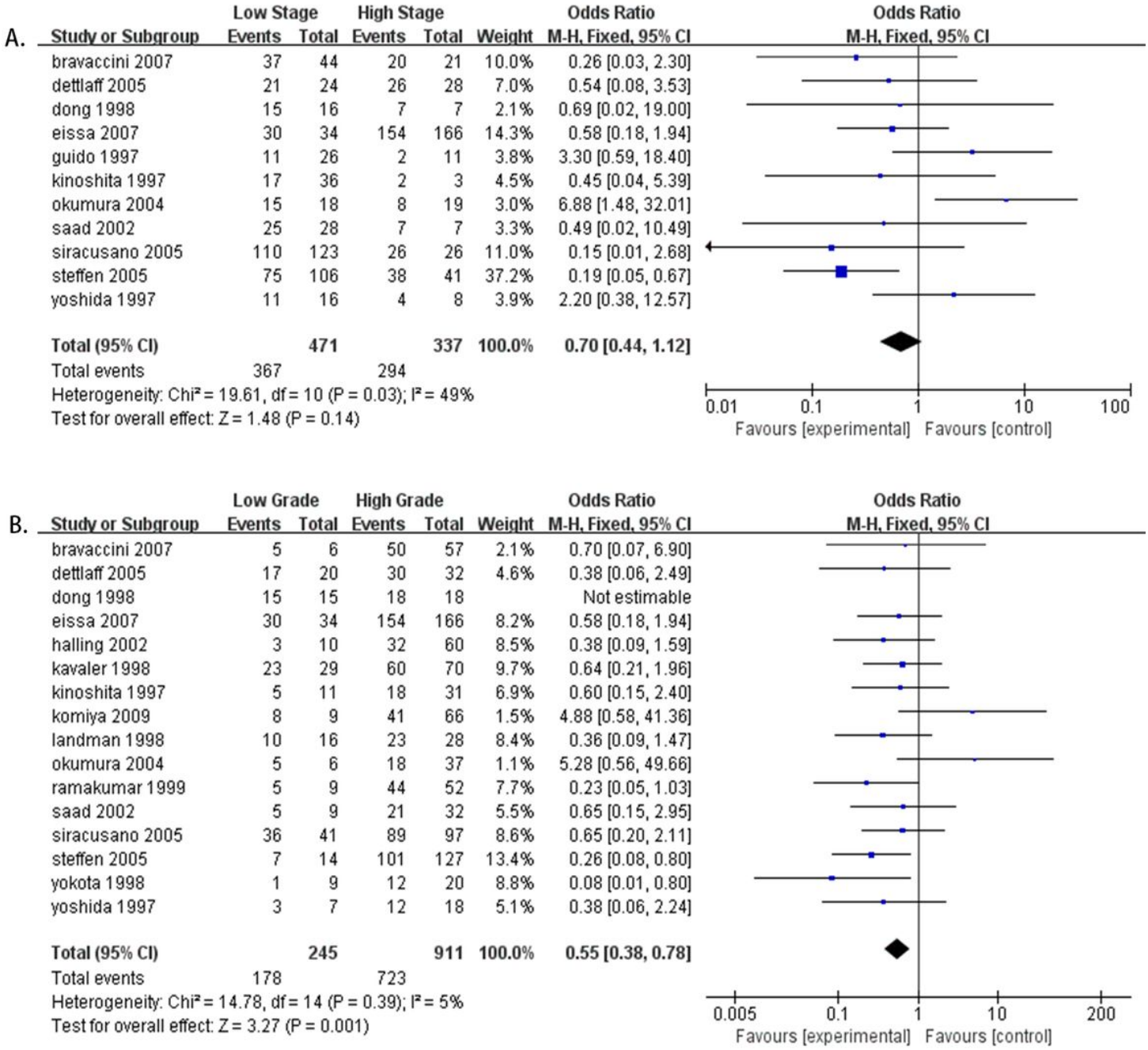

Figure 5

Forest plot depicting the pooled stages and grades for Telomerase Activity for Bladder Cancer. A: Forest plot for different stages; B: Forest plot for different grades

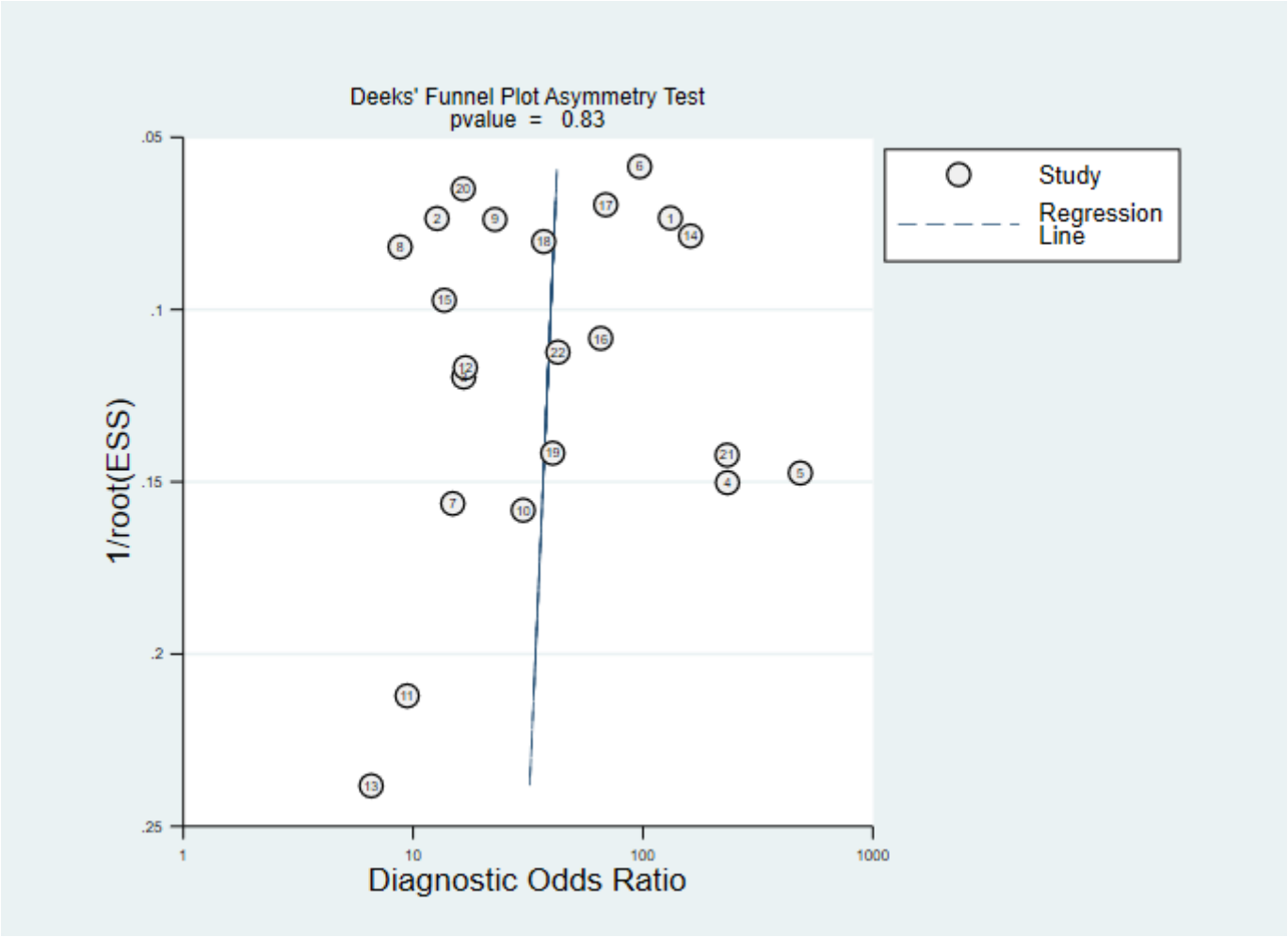

Figure 6

Deeks funnel plot to evaluate the publication bias.

Supplementary Files

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