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Review

Urinary *N*-Acetyl-β-D-glucosaminidase (uNAG) as an Indicative Biomarker of Early Diabetic Nephropathy in Patients with Diabetes Mellitus (T1DM, T2DM): A Systematic Review and Meta-Analysis

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Abstract: Diabetic nephropathy (DN) is the main cause of chronic kidney disease in patients with type 1 (T1DM) and type 2 diabetes mellitus (T2DM). Renal tubular lysosomal enzyme activities like *N*-acetyl-β-D-glucosaminidase (NAG) have been shown to increase in patients developing DN. The aim of this systematic review and meta-analysis is to evaluate the diagnostic accuracy of NAG, as a preventional biomarker in the early stages of DN in patients with diabetes mellitus. Two impartial reviewers conducted a complete PubMed search until July 2021. A 2 × 2 contingency table was created for each trial and sensitivity and specificity were estimated using a bivariate random effects model. To pool data and estimate the area under the curve (AUC), the hierarchical summary ROC (hsROC) approach was utilized. Deek's test was used to estimate publication bias. The meta-analysis included 21 studies that evaluated 2783 patients with T1DM and T2DM, as well as 673 healthy individuals. The AUC of urinary NAG (uNAG) ranged from 0.69 (95% CI: 0.65–0.73) to 0.89 (95% CI: 0.86–0.92). According to the results, NAG in urine can be considered as a potential and effective biomarker for predicting DN in diabetic patients (T1DM, T2DM).

Keywords: *N*-acetyl-β-D-glucosaminidase (NAG); diabetic nephropathy; chronic kidney disease (CKD); meta-analysis; systematic review

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1. Introduction

Diabetic nephropathy (DN), is a metabolic disease and one of the most frequent microvascular complications of type 1 (T1DM) and type 2 (T2DM) diabetes mellitus [1]. The prolonged exposure of the body to high blood glucose levels (hyperglycemia) due to diabetes, affects proper functioning of the kidneys by damaging specific units responsible for removing waste products from the body and filtering essential substances to pass into the bloodstream [1]. DN is independently associated with cardiovascular risk in diabetic patients, especially in patients with T2DM [2]. Therefore, early detection and treatment is of major importance, as it can prevent critical complications of the disorder. DN is the leading cause of chronic kidney disease (CKD) and its diagnosis is based on the current level of albuminuria leading in three stages [3]: DN normoalbuminuria, microalbuminuria, and macroalbuminuria. More specifically, the confirmation of the disease is based on the persistent albuminuria in early morning urine samples, due to glomerular hyperfiltration [4,5]. Healthy individuals excrete small amount of albumin on a daily basis which does not exceed 30 mg/g. Albumin-to-creatinine ratio (ACR) is the gold standard method to detect elevated protein excretion in urine samples of diabetic patients. The onset stage of DN is defined by moderately increased albuminuria, known as microalbuminuria and it is diagnosed by the detection of a significant amount of albumin in the urine, which ranges from 30–300 mg/24 h [5]. The progression of the disorder refers to a gradual decline in

GFR and is characterized by severely abnormal increased levels of albuminuria, known as macroalbuminuria (proteinuria). It is increasingly appreciated that both glomerular and tubular interstitial damage have an essential role in the pathophysiology and development of DN [6].

However, according to recent studies, in 30% of diabetic population diagnosed with microalbuminuria, the course of the disease has shifted to a new clinical picture, defined by normal albuminuria and GFR after 10 years of follow up [7–9]. Even though microalbuminuria measurement is the gold standard method to predict and monitor the progression of DN, significant efforts have been made to investigate and validate alternative biomarkers for the diagnosis of DN, allowing the early identification of diabetic renal lesions [10]. Multiple biomarkers have been reported and classified due to their ability of detecting specific disorders [11]. Studies have shown promising preliminary results, suggesting that increased levels of the potential biomarkers are associated with the presence of renal damage in patients with T1DM and T2DM. According to studies, some of the effective biomarkers of glomerular injury are: adiponectin [12,13], transferrin [14], and ceruloplasmin [15]. Studies have also shown potential biomarkers which reflect tubular injuries, these include kidney injury molecule-1 KIM-1, a1- and b2-microglobulin [16–18], liver-type fatty acid binding protein L-FABP [17], and neutrophil gelatinase-associated lipocalin (NGAL). Recent meta-analysis suggests that NGAL is a potential valuable biomarker for early prediction of DN in diabetic patients [19].

In addition, *N*-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme found in proximal renal tubular cells and its significant concentrations during tubulointerstitial damage, are related with renal dysfunction [20]. In previous studies, increased urinary levels of NAG concentrations were present in diabetic patients diagnosed with normoalbuminuria rather than the control group. In addition, urinary NAG (uNAG) has been shown to increase progressively along with the DN stages, indicating that it might be an early predictive biomarker for DN [21,22]. Thus, the aim of this study is to conduct a systematic review and meta-analysis to evaluate the diagnostic accuracy of uNAG, as a preventional biomarker in the early stages of DN in patients with diabetic mellitus.

2. Materials and Methods

2.1. Search Strategy and Study Selection

This systematic review and meta-analysis was conducted according to the standard PRISMA (Preferred Reporting Item for Systematic Reviews and Meta-analyses) guidelines [23]. A literature search was performed on the PubMed database by two independent reviewers until the end of July 2021, using a clearly formulated query of terms and keywords ("urinary biomarker" OR "N-Acetyl-beta-D-Glucosamine" OR "GLcNAc" OR "N-AcetylGlucosamine" OR "urinary NAG" OR "serum NAG" OR "urinary lysosomal enzyme") AND ("diabetes" OR "diabetic nephropathy" OR "diabetic kidney disease"). To eliminate local literature bias, the study search was comprehensive and did not include language limitations. In addition, further search was conducted in other electronic engines, such as Google Scholar, the duplicates records were removed. The study selection was based on specific predefined criteria and each reason for inclusion or exclusion was recorded. All of the studies were included after reviewing the abstracts and full text of each article.

2.2. Inclusion and Exclusion Criteria

Studies chosen for meta-analysis were based on specific inclusion criteria. The rationale for the criteria of the study selection was predefined and clearly stated. The meta-analysis included studies, in which uNAG was determined in healthy individuals and in patients with diabetes mellitus. Diabetic patients were divided in the three following categories: patients with normoalbuminuria (UACR < 30 mg/g), patients with microalbuminuria (UACR = 30–300 mg/g), and patients with macroalbuminuria (UACR > 300 mg/g). Studies eligible for the meta-analysis also included the degree of DN determined by the

estimation of the UACR using a 24-h urine sample, or a random morning urine sample, according to the American Diabetes Association [23].

2.3. Data Extraction

NAG concentration in urine samples and uNAG concentration normalized to the urinary creatinine (uNAG/Cr), were extracted from each study. Furthermore, data synthesis included the extraction of the first author's name, study location, year of publication, age and sex of the participant groups, type of diabetes and clinical characteristics of each study group. Finally, a 2 × 2 contingency table was constructed using the absolute data of true positive (TP), false positive (FP), true negative (TN), and false negative (FN) for comparing and combining the effects of different research.

2.4. Quality Assessment of Safety Studies

The study selection was performed guided by the mentioned criteria, followed by an in-depth quality assessment, using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS–2) [24]. The QUADAS tool consists of 14 questions of four different key domains. The risk of bias and the applicability of the studies were examined for each domain. The following judgements were used to complete the signaling questions: low, high, or unclear risk. For the quality analysis, the Review Manager Software (RevMan 5.4) was used.

2.5. Meta-Analysis

A random effects meta-analysis was performed in order to synthesize quantitative information from related studies. The approach method of the bivariate meta-analysis involves estimating sensitivity (logit-Se) and specificity (logit-Sp) by van Houwelingen [25,26].

According to the available raw data, the following statistical calculations were used for estimating the mean and *SD*. Therefore, in the studies where the 95% confidence interval (*CI*) was specified, *SE* was determined according to the following equation recommended by Cochrane Handbook [22]:

$$SE = (upperlimit - lowerlimit)/3.92 \quad (1)$$

In the studies where median (*M*) and inter-quartile range (*IQR*) were provided, for calculating mean and *SD* we followed the Cochrane Handbook [27]. The median was utilized as a mean estimator, while the *SD* was determined as follows:

$$SD = \frac{IQR}{1.35} \quad (2)$$

In the studies where median (*M*) and range were provided, we used the principles specified by Hozo and co-workers [28]. The following equation was used for sample sizes $n < 25$:

$$\overline{x} = \frac{\min + 2M + max}{4} \quad (3)$$

In the studies where the sample size was $n > 25$, median was chosen as the appropriate value over the mean value. *SD* for sample sizes $n < 15$ was calculated by the following equation:

$$SD^2 = \frac{1}{12}\left(\frac{(min + 2M + max)^2}{4} + (max - min)^2\right) \quad (4)$$

while, for $n > 25$ was calculated by the equation:

$$SD = \frac{R}{4} \quad (5)$$

The three following groups of diabetic patients were considered in the meta-analysis: patients with normoalbuminuria, patients with microalbuminuria, and patients with

normo/microalbuminuria. Normoalbuminuria was defined using as status variables healthy individuals (controls) vs. normoalbuminuric patient group whereas for the prediction of microalbuminuria, normoalbuminuric vs. microalbuminuric patients, patient groups were used as status variables.

In order to assess the diagnostic performance of uNAG and uNAG/Cr in early diagnosis of DN, the hierarchical summary ROC curve (hsROC) was constructed using sensitivity, specificity, and parameters of the bivariate normal distribution. For each study, the absolute number of TPs, FPs, FNs, and TNs were computed by altering the threshold values (log cutoff) calculated by the raw extracted data from the articles, such as the mean and standard deviation (*SD*) of uNAG and uNAG/Cr, assuming a normal distribution. The Youden index at its maximum value, represents the ideal discrimination limit, which is calculated as Y = sensitivity + specificity − 1 [29].

The interpretation of the curve was based on the following principals proposed by Swets [30]: Low ($0.5 \geq AUC \leq 0.7$), moderate ($0.7 \geq AUC \leq 0.9$), and high ($0.9 \geq AUC \leq 1.0$) accuracy. The between-study heterogeneity was estimated by using the Cochran Q-test and I^2 statistic and was presented as a forest plot [31]. For the publication bias were used calculation methods according to Deek's et al. [32]. The current study's data synthesis and statistical analysis were carried out using Stata software v.13 (College Station, TX, USA: StataCorp LLC).

3. Results

3.1. Included Studies and Trial Characteristics

Literature search from the databases yielded 353 citations, of which 3 were duplicates and discarded, resulting in 350 unique citations. Following the first review of the titles and abstracts, 283 articles were excluded for not meeting the inclusion criteria. Further, following a detailed examination of the 69 full-text articles, 48 articles were removed due to misclassification of DN or the unclear statement of the methods used in the study. Consequently, a total of 21 studies were found eligible and were included in the meta-analysis. The PRISMA flow of the review process is shown in Figure 1. These studies consisted of 2783 patients and 673 healthy in total. Table 1 represents the population's characteristics extracted from each study. Precisely, studies included 1196 patients with T1DM, 1587 patients with T2DM, and 673 healthy individuals. The group of patients with T1DM involved 644 patients (53.8%) with normoalbuminuria, 477 patients with microalbuminuria (39.8%), and 75 patients (6.3%) with macroalbuminuria. The group of patients with T2DM involved 760 patients (47.8%) with normoalbuminuria, 663 patients (41.7%) with microalbuminuria, and 164 patients (10.3%) with macroalbuminuria. Patients with macroalbuminuria were not included in the meta-analysis due to the insufficient number of studies. Furthermore, 50.5% of the patient group, were male with a mean age of 53.4 years old. In the healthy group 50.1% were male with a mean age of 51.3 years old. The clinical features collected from the studies are expressed by the weighted average and the concentrated standard deviation and are presented in supplementary Table S1.

Table 1. Detailed characteristics of the included studies in the meta-analysis for controls and diabetic patients.

	Controls			Diabetic Patients												
				Normoalbuminuria			Microalbuminuria			Macroalbuminuria						
Country	Sample Size (*n*)	Sex (%.Male/Female)	Age (Mean)	Sample Size (*n*)	Sex (%.Male/Female)	Age (Mean)	Sample Size (*n*)	Sex (%.Male/Female)	Age (Mean)	Sample Size (*n*)	Sex (%.Male/Female)	Age (Mean)	NAG Type	Determination Method of NAG	Data	Reference
Ghana	65	44.6/55.4	51.2	39	–		26	–	–	–	–	–	uNAG	Spectophotometric *	Mean, *SD*	[33]
Ghana	65	44.6/55.4	54	39	–		26	–	–	–	–	–	uNAG/Cr	Spectophotometric	Mean, *SD*	[33]
Iran	25	60/40	55.2	24	62.5/37.5	58.2	8	62.5/37.5	53.1	–	–	–	uNAG/Cr	Immunoturbidimetry	Mean, *SD*	[34]
Egypt	40	40/60	15.1	48	–	14.6	11	–	16.8	–	–	–	uNAG/Cr	Colorimetricanalysis **	Mean, *SD*	[35]
Poland	42	28.5/71.5	56	14	–	–	89	–	–	27	–	–	uNAG/Cr	Spectophotometric	Median *IQR*	[36]
India	48	–	45.6	94	–	–	102	–	–	–	–	–	uNAG/Cr	ELISA **	Mean, *SD*	[37]
Egypt	20	60/40	51	20	50/50	51.3	25	44/56	52.9	25	48/42	51.7	uNAG	Spectophotometric	Median *IQR*	[38]
Poland	32	37.5/62.5	61.9	29	38/62	63.4	32	34.3/65.7	63.4	29	34.5/65.5	62.4	uNAG	ELISA	Mean, *SD*	[39]
USA	38	50/50	43	363	44/56	39	296	61/39	41	–	–	–	uNAG	Spectophotometric	Mean, *SD*	[40]
Egypt	10	60/40	47.3	10	80/20	51.36	20	50/50	48.6	20	40/60	52.8	uNAG	ELISA	Mean, *SD*	[41]
Japan	57	59.6/40.4	44.5	90	–	47.5	–	–	–	–	–	–	uNAG/Cr	RIA	Mean, *SD*	[42]
India	48	–	45.3	94	–	–	102	–	–	–	–	–	uNAG/Cr	Spectophotometric	Mean, *SD*	[37]
Japan	–	–	–	20	45/55	57.1	17	35.2/64.8	62.7	–	–	–	uNAG/Cr	–	Median *IQR*	[43]
Spain	32	46.8/53.2	60	25	52/48	60	60	48.3/51.7	59	75	48/52	64	uNAG	Colorimetric analysis	Median *IQR*	[44]
Japan	20	55/45	57	19	84.2/15.8	62	7.8	18/82	72.2	19	56.2/43.8	60	uNAG	Colorimetric analysis	Median *IQR*	[45]
UK	20	50/50	45	20	–	–	20	–	–	–	–	–	uNAG	EIA	Mean, *SD*	[46]
UK	15	–	48	12	58.3/41.7	48	12	41.7/58.3	48	12	50/50	48	uNAG	EIA	Mean, *SD*	[47]
China	28	46.4/53.6	48.3	61	–	–	24	–	–	16	–	–	uNAG	Colorimetric analysis	Median *IQR*	[48]
Italy	31	32.2/67.8	61.1	43	37.1/62.9	64.2	–	–	–	–	–	–	uNAG	Colorimetric analysis	Median *IQR*	[49]
China	42	54.8/45.2	54.3	144	57.6/42.4	54.3	94	55.3/44.7	55.49	49	57.1/42.9	59.2	uNAG	Immunonephelometric	Median *IQR*	[50]
Skopje	30	66.6/33.4	33	170	56.4/43.6	50	115	56.5/43.5	57.3	–	–	–	uNAG	–	Mean, *SD*	[51]
Egypt	30	50/50	51	26	39/61	51	30	53/47	57	30	53/47	56	uNAG	ELISA	Mean, *SD*	[52]

* NAG absorbance for spectophotometric analysis: OD = 400–405 nm ** NAG absorbance for ELISA/colorimetric analysis: OD = 400 nm.

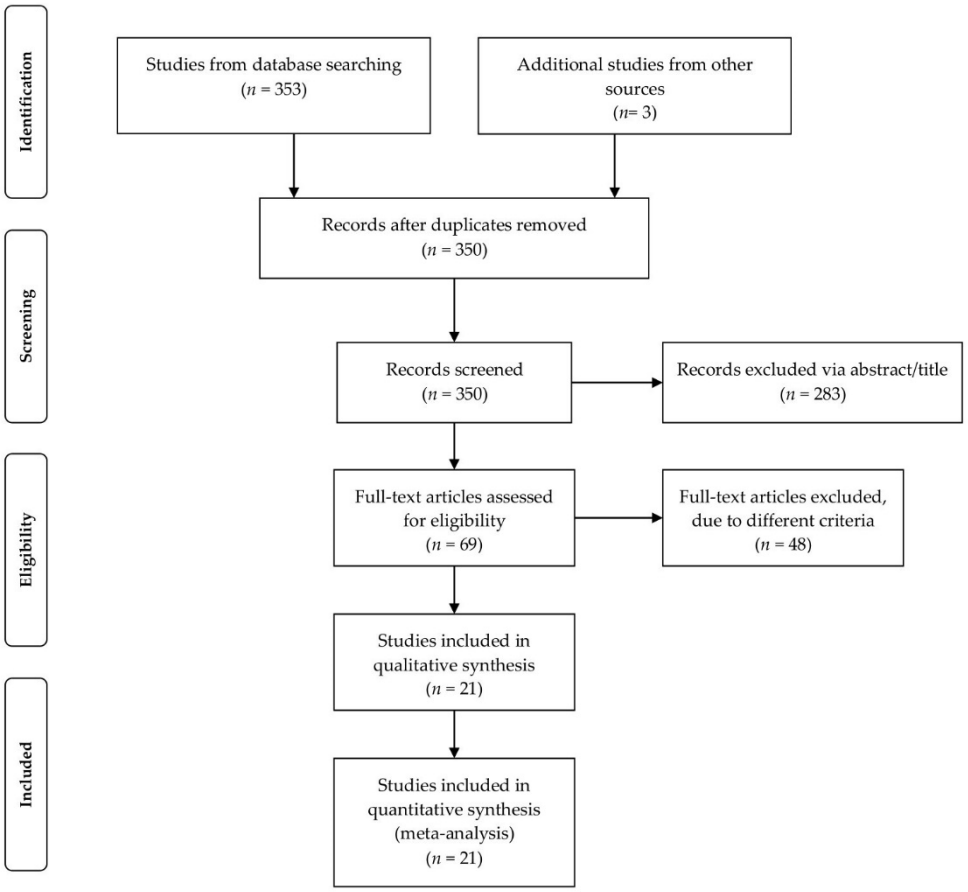


Figure 1. PRISMA flow diagram for literature search and study selection.

3.2. Quality Assessment of the Included Studies

The outcome of the comprehensive quality assessment of the 21 included studies are shown in the Figure 2. The unclear risk of bias in some studies on patient selection was present due to lack of information about the characteristics of the patient group with microalbuminuria and macroalbuminuria, such as the total number of patients, age, and gender. There was a concern regarding the applicability of the index test due to the different processing of the uNAG sample.

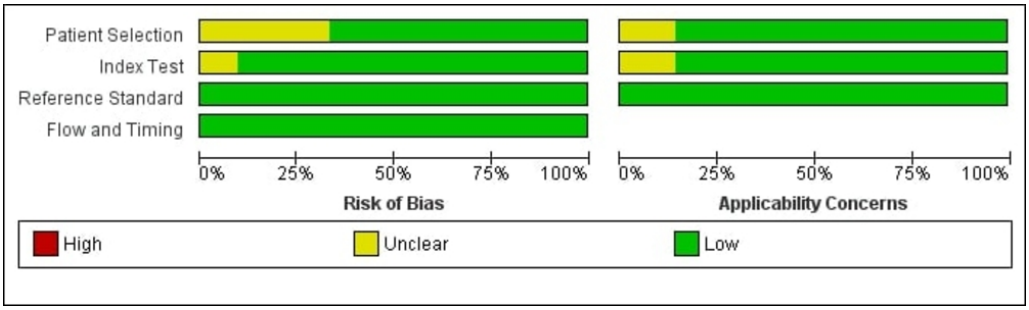
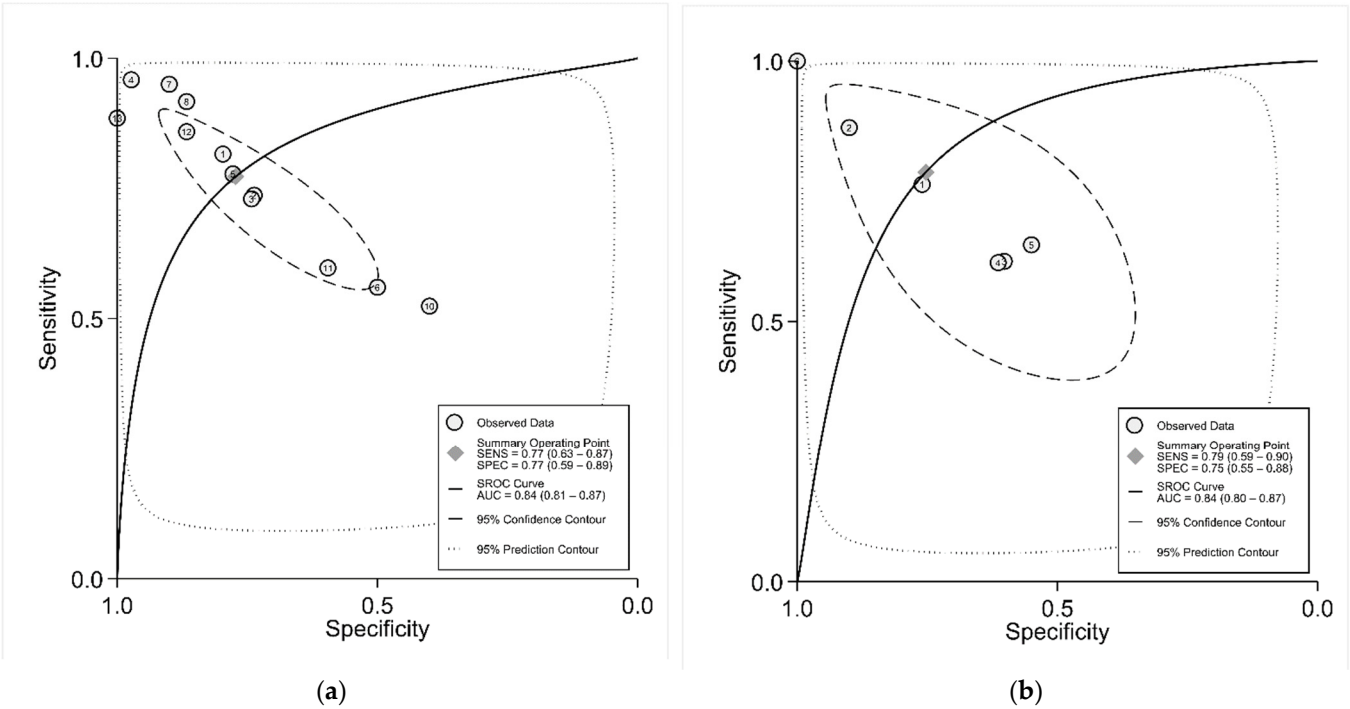


Figure 2. The Quality Assessment of Diagnostic Accuracy Studies–QUADAS. The figure represents the risk of bias and the applicability concern for the included studies. Each risk of bias is illustrated as percentage (%).

3.3. Diagnostic Accuracy and Summary ROC Curve

Overall, pooled sensitivity and specificity in diabetic patients, ranged from 0.65 (95% CI: 0.38–0.85) to 0.84 (95% CI: 0.77–0.89) and 0.65 (95% CI: 0.41–0.83) to 0.88 (95% CI: 0.67–0.97) respectively. An hsROC curve was created for each category (uNAG, uNAG/Cr)

and the AUC was calculated, along with the 95% CI. The diagnostic accuracy of uNAG estimated by AUC for predicting DN in diabetic patients, for all groups ranged from 0.69 (95% CI: 0.65–0.73) to 0.89 (95% CI: 0.86–0.92) (Figure 3 and supplementary Figures S1–S2). These results show moderate to excellent diagnostic accuracy of uNAG and uNAG/Cr. In addition, the best predictive performance was shown by uNAG and uNAG/Cr to discriminate between diabetic patients with normo-microalbuminuria and the healthy group with an AUC = 0.89 (95% CI: 0.86–0.92) (Figure 3). In the meta-analysis were used the following values, which are presented in Table 2: TP, FN, FP, and TN, paired sensitivity and specificity, along with the corresponding 95% CI and the cutoff values for each individual study. Table 3 provides the diagnostic and prognostic values for uNAG and uNAG/Cr.



(**a**) (**b**)

Figure 3. The hierarchical summary Receiver Operating Characteristic (hsROC) curve of uNAG (**a**) and uNAG/Cr (**b**) to differentiate controls (healthy individuals) from normoalbuminuric diabetic patients.

Table 2. Contingency table for uNGAL and uNGAL/Cr in diabetic patients, along with paired sensitivity and specificity of individual studies.

uNAG: Controls vs. Patients with Normoalbuminuria											
PubMed ID	**Author Name**	**Country**	**Year**	**Type of Diabetes**	**Cut-Off**	**TP ***	**FN ***	**TN ***	**FP ***	**Sensitivity (95%.CI)**	**Specificity (95% CI)**
27594733	Anane H.A.	Ghana	2016	2	11.15	31	7	51	13	0.80 (0.63–0.90)	0.79 (0.68–0.88)
23966807	Heba S. Assal	Egypt	2013	2	8.25	14	5	14	5	0.72 (0.48–0.90)	0.72 (0.48–0.90)
25519006	Zurawska Plaksej E.	Poland	2014	2	156.5	27	10	23	8	0.73 (0.56–0.86)	0.71 (0.53–0.86)
20980978	Vaidya S. V.	USA	2011	1	1.15	347	15	36	1	0.95 (0.92–0.97)	0.96 (0.86–0.99)
25717442	Gehan S.	Egypt	2015	2	1	7	2	7	2	0.70 (0.34–0.93)	0.70 (0.34–0.93)
16935891	Navarro J.F.	Spain	2006	1	1	14	11	16	16	0.56 (0.34–0.76)	0.50 (0.32–0.68)
17910281	Kalansoopiya A.	UK	2007	2	1	19	1	18	2	0.95 (0.75–0.99)	0.90 (0.68–0.98)
18236735	Kalansoopiya A.	UK	2007	2	1	11	1	13	2	0.91 (0.61–0.99)	0.86 (0.60–0.98)
21779943	Fu W.	China	2011	2	1	11	49	3	24	0.18 (0.09–0.30)	0.11 (0.02–0.29)
26904288	Muro P.D.	Italy	2015	2	1	22	20	16	24	0.52 (0.36–0.68)	0.40 (0.24–0.56)
31218128	Zhang D.	China	2019	2	1	86	58	25	17	0.59 (0.51–0.67)	0.40 (0.25–0.56)
-	Nikolov G.	Skopje	2013	2	1	146	24	26	4	0.85 (0.79–0.90)	0.86 (0.69–0.96)
32601635	Shrouq F.A.H.	Egypt	2020	2	1	23	3	30	0	0.88 (0.69–0.97)	1.00 (0.88–1.00)
uNAG/Cr: Controls vs. Patients with Normoalbuminuria											
PubMed ID	**Author Name**	**Country**	**Year**	**Type of Diabetes**	**Cut-Off**	**TP**	**FN**	**TN**	**FP**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
27594733	Anane H.A.	Ghana	2016	2	9.2	22	17	38	27	0.56 (0.39–0.72)	0.58 (0.45–0.70)
23105632	Ambade V.	India	2006	1.2	6.5	68	26	32	16	0.72 (0.62–0.81)	0.66 (0.51–0.79)
15016173	Salem M. A. K.	Egypt	2002	1	4.6	38	10	31	9	0.79 (0.65–0.89)	0.77 (0.61–0.89)
2881186	Shimojo N.	Japan	1987	1	2.3	99	1	56	1	1.00 (0.95–1.00)	1.00 (0.95–1.00)
23105632	Ambade V.	India	2003	1	6.2	65	29	33	15	0.68 (0.53–0.81)	0.69 (0.58–0.78)
16641878	Piwowar A.	Poland	2006	2	0.3	9	17	27	35	0.34 (0.17–0.55)	0.43 (0.31–0.56)
18022929	Karakani A. M.	Iran	2007	1	3.6	23	1	24	1	1.00 (0.85–1.00)	1.00 (0.85–1.00)
uNAG: Patients with Normoalbuminuria vs. Patients with Microalbuminuria											
PubMed ID	**Author Name**	**Country**	**Year**	**Type of Diabetes**	**Cut-Off**	**TP**	**FN**	**TN**	**FP**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
27594733	Anane H.A.	Ghana	2016	2	12.9	2	1	1	1	0.53 (0.37–0.69)	0.52 (0.33–0.73)
23966807	Heba S. Assal	Egypt	2013	2	13.8	1	3	2	4	0.76 (0.50–0.91)	0.91 (0.73–0.99)
25519006	Zurawska Plaksej E.	Poland	2014	2	193.5	2	1	2	1	0.54 (0.38–0.71)	0.48 (0.29–0.65)
16966829	Fujita H.	Japan	2006	2	20	18	0	19	0	1.00 (0.81–1.00)	1.00 (0.82–1.00)
25717442	Gehan S.	Egypt	2015	2	1.2	6	3	12	7	0.62 (0.26–0.87)	0.60 (0.36–0.80)
16935891	Navarro J.F.	Spain	2006	1	4	34	26	14	11	0.56 (0.43–0.69)	0.56 (0.34–0.75)
21779943	Fu W.	China	2011	2	12.7	16	8	41	20	0.66 (0.44–0.84)	0.67 (0.54–0.78)
20980978	Vaidya S.V.	USA	2011	1	2.5	2	6	2	4	0.82 (0.78–0.86)	0.84 (0.79–0.88)

Table 2. *Cont.*

uNAG/Cr: Patients with Normoalbuminuria vs. Patients with Microalbuminuria										
PubMed ID	**Author Name**	**Country**	**Year**	**Type of Diabetes**	**Cut-Off**	**TP**	**FN**	**TN**	**FP**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
27594733	Anane H.A.	Ghana	2016	2	15	29	9	19	6	0.76 (0.60–0.88)	0.75 (0.56–0.91)
15016173	Salem M. A. K.	Egypt	2002	1	9.8	41	6	9	1	0.85 (0.72–0.93)	0.87 (0.58–0.99)
16641878	Piwowar A.	Poland	2006	2	1.1	8	5	53	35	0.57 (0.28–0.82)	0.60 (0.49–0.70)
23105632	Ambade V.	India	2003	1	9.6	57	36	62	39	0.61 (0.51–0.71)	0.60 (0.50–0.70)
16373913	Narita T.	Japan	2005	2	3	11	6	11	9	0.67 (0.38–0.85)	0.55 (0.31–0.76)
18022929	Karakani A. M.	Iran	2007	1	6.2	23	0	7	0	1.00 (0.85–1.00)	1.00 (0.85–1.00)
uNAG: Controls vs. Patients with Normo-Microalbuminuria										
PubMed ID	**Author Name**	**Country**	**Year**	**Type of Diabetes**	**Cut-Off**	**TP**	**FN**	**TN**	**FP**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
23966807	Heba S. Assal	Egypt	2013	2	10	38	7	19	1	0.84 (0.70–0.93)	0.93 (0.75–0.99)
25519006	Zurawska Plaksej E.	Poland	2014	2	160	53	17	23	9	0.75 (0.63–0.85)	0.72 (0.53–0.86)
20980978	Vaidya S. V.	USA	2011	1	1.3	597	62	38	0	0.90 (0.88–0.92)	1.00 (0.90–1.00)
25717442	Gehan S.	Egypt	2015	2	1	21	9	8	2	0.70 (0.50–0.85)	0.79 (0.44–0.97)
uNAG /Cr: Controls vs. Patients with Normo-Microalbuminuria										
PubMed ID	**Author Name**	**Country**	**Year**	**Type of Diabetes**	**Cut-Off**	**TP**	**FN**	**TN**	**FP**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
15016173	Salem M. A. K.	Egypt	2002	1	5.2	53	6	33	7	0.89 (0.79–0.96)	0.82 (0.67–0.92)
16641878	Piwowar A.	Poland	2006	2	0.5	55	48	17	25	0.53 (0.43–0.63)	0.40 (0.25–0.56)
18022929	Karakani A. M.	Iran	2007	1	4	32	0	25	0	1.00 (0.89–1.00)	1.00 (0.89–1.00)
23105632	Ambade V.	India	2003	1	6.5	142	54	34	14	0.72 (0.65–0.78)	0.70 (0.55–0.83)
27594733	Anane H.A.	Ghana	2016	2	11	45	20	44	21	0.69 (0.56–0.80)	0.68 (0.54–0.78)

* TP: True Positive, * FN: False Negative, * TN: True Negative, * FP: False Positive.

Table 3. Pooled diagnostic and prognostic accuracy of uNAG in T1DM and T2DM patients.

Number of Studies	Sensitivity (95% CI)	I^2 (%)	Specificity (95% CI)	I^2 (%)	PLR (95%CI)	NLR (95% CI)	DOR (95% CI)	AUC (95% CI)	*p*-Value
uNAG: Controls vs. patients with normoalbuminuria									
13	0.77 (0.63–0.87)	64.65 (37.83–91.46)	0.77 (0.59–0.89)	58.22 (25.48–90.96)	3.4 (1.5–7.6)	0.29 (0.14–0.06)	12 (3–52)	0.84 (0.81–0.87)	0.89
uNAG/Cr: Controls vs. patients with normoalbuminuria									
7	0.82 (0.56–0.94)	93.22 (89.64–96.80)	0.79 (0.57–0.92)	93.95 (90.87–97.04)	3.9 (1.4–11.1)	0.23 (0.07–0.79)	17 (2–159)	0.87 (0.84–0.90)	0.63
uNAG: Patients with normoalbuminuria vs. patients with microalbuminuria									
8	0.65 (0.38–0.85)	64.65 (37.83–91.46)	0.65 (0.41–0.83)	58.22 (25.48–90.96)	1.8 (0.7–4.8)	0.54 (0.20–1.49)	3 (0–24)	0.69 (0.65–0.73)	0.66
uNAG/Cr: Patients with normoalbuminuria vs. patients with microalbuminuria									
6	0.79 (0.59–0.90)	82.49 (69.37–95.61)	0.75 (0.55–0.88)	85.76 (75.66–95.87)	3.2 (1.4–7.4)	0.28 (0.11–0.70)	11 (2–61)	0.84 (0.80–0.87)	0.13
uNAG: Controls vs. patients with normo-microalbuminuria									
4	0.83 (0.73–0.89)	87.99 (78.95–97.04)	0.92 (0.66–0.99)	74.65 (51.70–97.59)	10.8 (1.9–61.9)	0.19 (0.11–0.33)	58 (6–540)	0.90 (0.88–0.93)	0.49
uNAG/Cr: Controls vs. patients with normo-microalbuminuria									
5	0.84 (0.56–0.95)	96.43 (94.53–98.32)	0.81 (0.48–0.95)	93.13 (88.69–97.56]	4.4 (1–19]	0.20 (0.05–0.85)	22 (1–388)	0.89 (0.86–0.92)	0.08

3.4. Subgroup Analysis and Publication Bias

The forest plot graphical presentation had shown notable heterogeneity in sensitivity and specificity in all sets examined. The degree of heterogeneity in sensitivity and specificity for all groups ranged from 64.65% to 96.43% and from 58.22% to 93.95%, correspondingly. The between study heterogeneity for the prediction of normoalbuminuria and microalbuminuria in diabetic patients is presented in supplementary Figures S3–S5. The Deek's funnel plot estimated the evaluation of publication bias, in which *p*-value for all the groups, ranged from 0.08 to 0.89 and is showed in supplementary Figures S6–S8. Potential bias was present in the studies with data involving uNAG, specifically for distinguishing controls and normoalbuminuric or microalbuminuric diabetic patients.

4. Discussion

Diabetic nephropathy is considered to be the main cause of end-stage renal disease and a critical complication in patients with T1DM and T2DM. Therefore, the prediction of the disease at an early stage of its development is considered of the highest value. The diagnosis of DN is based on microalbuminuria estimation [5]. However, according to recent studies, diabetic patients diagnosed with microalbuminuria, have shifted back to normoalbuminuria and high GFR [7–9]. In addition, pathogenesis of DN evolves an interaction between metabolic and hemodynamic factors which cause glomerular and tubular interstitial injury. Therefore, researchers have been questioning and reevaluating the diagnostic value of the gold standard method, proposing an alternative approach, using potential tubular biomarkers such as NAG, for the early prediction of DN or other glomerular and markers of oxidative stress or inflammation. The attempt of this systematic review and meta-analysis was to evaluate the diagnostic accuracy of uNAG and provide comprehensive information for the accuracy of uNAG, as a preventional biomarker in the early stages of DN in patients with T1DM and T2DM.

Anane et al. [33] in their research study showed that the values of uNAG and uNAG/Cr ratio from patients' urine samples, had an increasing rate as the values of albuminuria increased and the rate of glomerular infiltration (eGFR) decreased, in patients with T2DM compared to the control group. Sheira's et al. [41] study showed statistically significant increase of uNAG and uNAG/Cr ratio and decrease of the estimated GFR in patients with microalbuminuria compared to patients with normoalbuminuria and in all patient groups compared to control group. Moreover, a parallel increase of the urinary excretion of NAG with the deterioration of DN has been observed, which indicates the severity of kidney damage and disease progression [40,41]. Kim et al. in their study showed that the levels of urinary NAG had a moderate positive correlation with the levels of urinary ACR in T2DM and that increased levels in urinary NAG may be associated with glycemic parameters reflecting glucose fluctuation [53].

The main result of our meta-analysis is the high value of AUC for uNAG and uNAG/Cr in distinguishing the control group from normo-microalbuminuric diabetic patients. This finding shows that uNAG/Cr can be considered a potential, good biomarker to predict early diabetic nephropathy in patients with diabetes mellitus. Moreover, according to the guidelines by Swets, the diagnostic accuracy values of uNAG and uNAG/Cr showed moderate accuracy in the other settings as well. These findings support the hypothesis for renal tubule damage even in the initial stage stages of DN, before the presence of pathological amounts of albuminuria, indicating a promising diagnostic accuracy of the biomarker. It is worth mentioning that uNAG/Cr seemed to present higher capability than uNAG in the diagnosis of DN, as designated by the comparison of the corresponding AUC values for each group.

According to the forest plot, there was significant presence of heterogeneity between studies. Possible causes of heterogeneity were mainly due to (a) the design of the studies which indicated different methodology of estimating creatine's concentration, (b) the different choice of urine collection for estimating ACR (spot urine or 24 h urine collection), and (c) the disparate race and ethnicity of the study population. In addition, a significant

decrease in heterogeneity was observed among the category of diabetic patients with normoalbuminuria and diabetic patients with microalbuminuria. The low presence of heterogeneity was caused due to the common procedure followed in each study.

Furthermore, according to Deek's charts, publication bias was absent in the majority of studies. Publication bias was evident in studies that performed the discrimination between controls and normo-microalbuminuric diabetic patients using uNAG/Cr. This bias may be introduced due to unclear methodology used for estimating the values of microalbuminuria in some of the studies. As evidenced by the QUADAS quality assessment, the vague risk of bias in some studies on patient selection was present due to insufficient information about the diabetic patients' characteristics with normoalbuminuria and microalbuminuria.

In conclusion, the final results of this systematic review and meta-analysis indicate that uNAG is a promising biomarker (raw and creatinine-normalized) for early and valid prediction of diabetic nephropathy in patients with T1DM and T2DM. Meta-analysis findings indicate that uNAG/Cr has higher efficiency in all patient groups. In particular, higher accuracy was observed in identifying the presence of DN in normo-microalbuminuric patients with T1DM and T2DM.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/diabetology2040025/s1, Table S1. Clinical characteristics of the included studies in the meta-analysis for controls (healthy individuals) and diabetic patients with normoalbuminuria and microalbuminuria. Figures S1–S2. The hierarchical summary Receiver Operating Characteristic (hsROC) curve of uNAG and uNAG/Cr to discriminate normo-, micro-, normo/microalbuminuric diabetic patients. Figures S3–S5. Forest plot for sensitivity and specificity of uNAG and uNAG/Cr to distinguish normo-, micro-, normo/microalbuminuric diabetic patients. Figures S6–S8. Deek's funnel plot for the evaluation of publication bias of uNAG and uNAG/Cr.

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