Role of Serum Chromogranin A in Early Diagnosis of Diabetic Nephropathy

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Research

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Abstract

Background: Kidney is the main site for the removal of Chromogranin A (CgA). Previous studies have found that patients with renal impairment displayed elevated concentrations of CgA in plasma and the CgA concentrations reflect deterioration of renal function. In this study, we aimed to estimate the serum CgA levels and to evaluate the role of serum CgA in early diagnosis of diabetic nephropathy (DN).

Methods: A total of 219 patients with type 2 diabetes mellitus (T2DM) were included in this cross-sectional study. They were classified into normoalbuminuria (n = 121), microalbuminuria (n = 73), or macroalbuminuria (n = 25) groups based on their urine albumin to creatinine ratios (UACR). A control group consisted of 45 healthy subjects. The serum CgA levels were measured by ELISA and other key parameters were assayed.

Results: The serum CgA levels were higher in patients with T2DM compared to control subjects and a statistically significant difference among studied subgroups regarding CgA was found ($P < 0.05$). Levels of serum CgA were increasing gradually with the degree of DN ($P < 0.001$). Serum CgA levels were moderate intensity positively correlated with UACR ($P < 0.001$). A cut-off level of 3.46 ng/ml CgA showed 69.86% sensitivity and 66.12% specificity to detect DN in early stage.

Conclusions: Levels of serum CgA increased gradually with the degree of DN, and can be used as a biomarker in early detection of DN.

Introduction

Diabetes is one of the fastest growing health challenges of the 21st century. According to the recent estimates from the International Diabetes Federation (IDF), 463 million adults are currently living with diabetes. IDF estimates that there will be 578 million adults with diabetes by 2030, and 700 million by 2045. Globally, 11.3% of deaths are due to diabetes [1]. Diabetic nephropathy (DN) is a common and serious complication of diabetes and has been shown to be a major cause of end-stage renal disease (ESRD), requiring costly renal replacement therapy in the form of dialysis or transplantation [2]. It is appreciated that up to 40% of the patients with type 1 and type 2 diabetes mellitus (DM) present DN [3, 4]. Early detection and appropriate treatment are essential to prevent disability and death.

At present, the clinical diagnosis of DN relies mainly on the detection of urine microalbumin. Although it is a gold standard for early detection of DN, its predictive powers are still limited. In some cases, patients with type 2 diabetes mellitus (T2DM) have progressive loss of renal function before the onset of microalbuminuria. Thus, in patients with T2DM, microalbuminuria is not specific and sensitive enough for early detection of DN [5, 6]. Identification of new biomarkers are urgently warranted that can be used as alternative to –or together with– routine biomarkers in early detection of DN. In addition, new DN biomarkers may also provide new insight into the pathophysiological mechanisms leading to complications, which are still not fully understood.

Chromogranin A (CgA) is the main member of the chromogranins family, and is an acidic glycoprotein consisting of 439 amino-acids with an approximate molecular mass of 48 kDa. It is used as a diagnostic marker of neuroendocrine tumors and to monitor tumor progression or regression during treatment [7]. It also has a role as biomarker in neurodegenerative and neuropsychiatric diseases, hypertension, cardiovascular disease, heart failure and renal and liver failure [7, 8]. Kidney is the main site for the removal of CgA and it is retained in serum with declining renal function [9, 10]. In this study, we aimed to estimate the serum CgA levels and to determine the sensitivity and specificity of this biomarker for early detection of DN.

Materials And Methods

Patients and Samples

In this cross-sectional study, 219 patients with T2DM (per the 2007 American Diabetes Association diagnosis) who were hospitalized in the Department of Endocrinology, Putuo Hospital, Shanghai University of Traditional Chinese Medicine, China, between April 2018 and April 2019, were selected. This study was approved by the Ethics Committee of our hospital (Approval number PTEC-A-2018-11-1). All participants signed informed consent. The specific inclusion criteria were as follows: (1) aged ≥ 18 years; (2) Known diabetes duration ≥ 1 year; (3) $\text{eGFR} > 60 \text{ mL/min/1.73m}^2$, and $\text{UACR} \leq 300 \text{ mg/g}$; or $\text{eGFR} < 60 \text{ mL/min/1.73m}^2$, and $\text{UACR} > 300 \text{ mg/g}$. Patients with hepatic diseases, heart failure, thyroid disorders, autoimmune diseases, inflammatory conditions and sepsis, malignancy, renal impairment of known origin, urinary tract infections, past history of rapidly progressive renal failure, any type of glomerulonephritis, and patient with polycystic kidney, mental patients, pregnant or lactating women were excluded from the study. In the same period, 45 healthy people who did physical examination in our hospital were selected as controls. All groups were matched for age and sex.

All patients with T2DM were divided into three subgroups according to urinary microalbumin to creatinine ratio (UACR): 1. patients with normoalbuminuria – $\text{UACR} < 30 \text{ mg/g}$ (n = 121), 2. patients with microalbuminuria – $\text{UACR} 30\text{--}300 \text{ mg/g}$ (n = 73), 3. patients with macroalbuminuria – $\text{UACR} > 300 \text{ mg/g}$ (n = 25). Classification was performed according to guidelines of KDIGO – Kidney Disease: Improving Global Outcomes-guidelines from 2012 [11]. Increased albuminuria ($\text{UACR} \geq 30 \text{ mg/g}$) was considered the objective evidence of kidney disease.

UACR 30–300 mg/g and eGFR > 60 mL/min/1.73m² was considered as early DN; UACR > 300 mg/g and eGFR < 60 mL/min/1.73m² was considered as advanced DN.

Spot urine samples were collected at first morning void. Blood samples were collected before eating in the morning, they were centrifuged (3,000 rpm, 4 ° C, 10 min) to acquire serum and stored at – 80 ° C until use. Samples for measurements of serum creatinine (sCr), serum uric acid (sUA), blood urea nitrogen (BUN), fasting blood glucose (FBG), hemoglobin A1c (HbA1c), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), urinary microalbumin to creatinine ratio (UACR) levels were examined at the department of clinical examination using standard procedures. The estimated glomerular filtration rate (eGFR) was calculated using the abbreviated MDRD equation [12]. $eGFR \text{ (mL/min per } 1.73\text{m}^2) = 186 \times Pcr^{-1.154} \times age^{-0.203} \times 0.724$ (if female), where Pcr is in mg/dl and age is in years. The concentration of CgA was measured using Human-CgA ELISA kits (Cat# ab196271) from Abcam.

Statistical Analysis

Continuous variables with normal distribution were reported as mean ± standard deviation (SD), whereas skewed distributed variables were expressed as median (interquartile range). Categorical data were summarized as proportions with frequencies. Continuous variables were compared between groups using unpaired t test, one-way ANOVA test, Kruskal–Wallis test or Jonckheere–Terpstra test where appropriate, while the chi-square test or Fisher's exact test was used to analyze the differences in categorical variables. Logistic regression analyses were used to ascertain the predictive value of CgA level for the presence of early DN in patients with T2DM. Considering the interaction between sCr and eGFR, and their similar clinical implication, only sCr would be used for multivariate logistic regression. Spearman rank correlation test was performed to analyze the relationship between 2 quantitative parameters. Statistical analyses were conducted using SPSS software (version 23.0; SPSS, Inc., Chicago, IL, United States). Statistical significance was considered as 2-tailed, with a p-value of < 0.05.

Results

Clinical Characteristics

All enrolled T2DM patients were categorized into three subgroups according to UACR. Demographic and laboratory features of the patients as well as the healthy subjects are reported in Table 1. Our results showed that there was significant difference among the subgroups of patients regarding to SBP, Known diabetes duration, UACR, sCr, BUN and eGFR. Meanwhile, there was no significant difference among the subgroups of patients regarding to gender, age, DBP, FBG, HbA1c, sUA, TC, TG, HDL-C and LDL-C. Compare the clinical and laboratory data among the subgroups of patients and the healthy subjects, there was no significant statistical difference only in gender, age, TC and LDL-C levels.

Table 1
Comparison of clinical and laboratory data among subgroups of patients with T2DM divided according to UACR and healthy subjects

	Heathy controls (n = 45)	Normo Albuminuria (n = 121)	Micro albuminuria (n = 73)	Macro albuminuria (n = 25)	^a p- value	^b p- value
Male,n(%)	26(57.8%)	72(59.5%)	38(52.1%)	15(60.0%)	0.768	0.568
Age(years)	63.40 ± 10.38	62.51 ± 10.34	65.18 ± 13.52	64.48 ± 10.85	0.445	0.277
SBP(mmHg)	117.44 ± 7.04	129.50 ± 12.15 ^{&&}	132.12 ± 14.41 ^{&&}	141.60 ± 17.68 ^{&&***#}	< 0.001	< 0.001
DBP(mmHg)	74.00 ± 6.79	79.41 ± 7.76 ^{&&}	78.96 ± 9.47 ^{&}	79.88 ± 6.70 ^{&}	0.001	0.874
FBG(mmol/L)	4.81 ± 0.42	8.63 ± 4.14 ^{&&}	8.79 ± 3.50 ^{&&}	9.10 ± 2.76 ^{&&}	< 0.001	0.850
HbA1c(%)	5.50 ± 0.37	9.41 ± 2.40 ^{&&}	9.88 ± 3.19 ^{&&}	9.45 ± 2.17 ^{&&}	< 0.001	0.479
Known diabetes duration (years)	/	8.00(3.00,15.00)	10.00(6.00,20.00) ^{**}	18.00(13.00,20.00) ^{**#}	/	< 0.001
UACR(mg/g)	3.59(2.04,5.75)	8.55(5.30,14.07) ^{&&}	77.69(48.49,166.00) ^{&&***}	834.05(614.33,2103.99) ^{&&***##}	< 0.001	< 0.001
sCr(umol/L)	66.73 ± 12.19	65.07 ± 13.82	72.07 ± 18.48 [*]	137.04 ± 49.66 ^{&&***##}	< 0.001	< 0.001
BUN(mmol/L)	5.11 ± 0.96	5.58 ± 1.45	7.08 ± 2.81 ^{&&***}	8.91 ± 4.33 ^{&&***#}	< 0.001	< 0.001
sUA(ummol/L)	308.84 ± 71.81	325.74 ± 94.77	345.77 ± 101.29 ^{&}	373.84 ± 103.01 ^{&*}	0.022	0.060
eGFR(ml/min/1.73m ²)	102.44 ± 21.80	106.44 ± 21.12	95.31 ± 28.75 [*]	47.53 ± 12.50 ^{&&***##}	< 0.001	< 0.001
TC(mmol/L)	4.67 ± 0.72	4.68 ± 1.59	4.38 ± 1.22	4.41 ± 1.38	0.421	0.335
TG(mmol/L)	0.94(0.75,1.33)	1.55(1.08,2.43) ^{&}	1.40(0.99,2.12) ^{&}	1.66(1.13,2.63) ^{&}	< 0.001	0.360
LDL-C(mmol/L)	2.97 ± 0.84	2.93 ± 1.15	2.69 ± 0.84	2.84 ± 1.00	0.373	0.319
HDL-C(mmol/L)	1.41 ± 0.28	1.13 ± 0.35 ^{&&}	1.11 ± 0.31 ^{&&}	1.06 ± 0.27 ^{&&}	< 0.001	0.660
CgA(ng/mL)	1.89(1.42,2.47)	2.26(1.38,4.12) ^{&}	4.83(2.63,6.46) ^{&&***}	6.35(4.91,7.54) ^{&&***#}	< 0.001	< 0.001
Notes:						
& P < 0.05.						
&& P < 0.001 vs. healthy controls.						
* P < 0.05.						
** P < 0.001 vs. normoalbuminuria group.						
# P < 0.05.						
## P < 0.001 vs. microalbuminuria group.						
^a P-value: comparisons among the healthy controls group, the normoalbuminuria group, the microalbuminuria group and the macroalbuminuria group;						
^b P-value: comparisons among the normoalbuminuria group, the microalbuminuria group and the macroalbuminuria group						
SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; UACR, urine albumin to creatinine ratio; sCr, serum creatinine; BUN, blood urea nitrogen; sUA, serum uric acid; eGFR, estimated glomerular filtration rate; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; CgA, Chromogranin A.						

Comparison of serum CgA levels among subgroups of patients with T2DM and healthy subjects

A comparison of serum CgA levels among subgroups of patients divided according to UACR and healthy subjects was performed using the Kruskal-Wallis test. The serum CgA levels were higher in patients with T2DM compared to healthy subjects and a statistically significant difference among studied subgroups regarding CgA was found ($P < 0.05$). A non-parametric Jonckheere-Terpstra test showed that CgA was increasing gradually with the degree of DN ($Z = 6.567$, $P < 0.001$). The results are shown in Fig. 1 and Table 1.

Correlation between serum CgA levels and clinical and laboratory data

Spearman rank correlation test demonstrated that there was moderate intensity positive correlation between serum CgA levels and Known diabetes duration, UACR in all subjects ($P < 0.001$). Weak correlation was found between serum CgA levels and FBG, HbA1c, sCr, BUN, eGFR, HDL-C (all $P < 0.05$), and there was no correlation between serum CgA levels and age, SBP, DBP, sUA, TC, TG, LDL-C in all subjects. There was moderate intensity positive correlation between serum CgA levels and UACR in patients with T2DM ($P < 0.001$). Weak correlation was found between serum CgA levels and Known diabetes duration, DBP, HbA1c, sCr, BUN, eGFR (all $P < 0.05$), and there was no correlation between serum CgA levels and age, SBP, FBG, sUA, TC, TG, LDL-C, HDL-C in patients with T2DM. The correlation coefficients between serum CgA levels and clinical and laboratory data are presented in Table 2.

Table 2
Correlation between serum CgA and clinical and laboratory data of subjects

clinical and laboratory data	CgA (in all subjects) (n = 264)		CgA (in patients with T2DM) (n = 219)	
	r	P	r	P
Known diabetes duration (years)	0.302	< 0.001	0.171	0.011
Age(years)	0.066	0.286	0.053	0.434
SBP(mmHg)	0.099	0.110	-0.029	0.673
DBP(mmHg)	-0.084	0.172	-0.170	0.012
FBG(mmol/L)	0.240	< 0.001	0.128	0.060
HbA1c(%)	0.285	< 0.001	0.144	0.033
UACR(mg/g)	0.441	< 0.001	0.408	< 0.001
sCr(umol/L)	0.163	0.008	0.188	0.005
BUN(mmol/L)	0.229	< 0.001	0.202	0.003
sUA(ummol/L)	0.061	0.322	0.049	0.470
eGFR(ml/min/1.73m ²)	-0.227	< 0.001	-0.252	< 0.001
TC(mmol/L)	-0.118	0.056	-0.109	0.107
TG(mmol/L)	0.035	0.572	-0.049	0.473
LDL-C(mmol/L)	-0.109	0.076	-0.105	0.120
HDL-C(mmol/L)	-0.124	0.044	-0.027	0.689

SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; UACR, urine albumin to creatinine ratio; sCr, serum creatinine; BUN, blood urea nitrogen; sUA, serum uric acid; eGFR, estimated glomerular filtration rate; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; CgA, Chromogranin A.

Serum CgA level was associated with the occurrence of early DN

We performed multivariate logistic regression analyses in patients with T2DM between the normoalbuminuria group and the microalbuminuria group. As shown in Table 3, multivariable logistic regression analysis revealed that serum CgA (OR 1.403, 95%CI 1.202–1.637, $P < 0.001$) and BUN (OR 1.299, 95%CI 1.045–1.616, $P = 0.019$) were independently associated with the occurrence of early DN, when adjusted for age, gender, Known diabetes duration, SBP, DBP, FBG, HbA1c, TC, TG, HDL-C, LDL-C, sUA, BUN, sCr and CgA. (Enter)

Table 3

Multivariate logistic regression analysis of factors independently associated with early DN in patients with T2DM between the normoalbuminuria group and the microalbuminuria group.

Variables	P-value	EXP(B)	95% CI
Male	0.252	0.603	0.254–1.434
Age	0.375	0.983	0.946–1.021
Known diabetes duration	0.164	1.039	0.985–1.096
SBP	0.468	1.013	0.978–1.049
DBP	0.538	1.017	0.963–1.074
FBG	0.564	0.971	0.877–1.074
HbA1c	0.519	1.050	0.905–1.219
TC	0.686	0.850	0.386–1.871
TG	0.637	1.052	0.851–1.302
HDL-C	0.315	2.176	0.478–9.917
LDL-C	0.927	0.958	0.385–2.384
sUA	0.816	0.999	0.994–1.004
BUN	0.019	1.299	1.045–1.616
sCr	0.097	1.029	0.995–1.064
CgA	< 0.001	1.403	1.202–1.637
adjusting for age, gender, Known diabetes duration, SBP, DBP, FBG, HbA1c, TC, TG, HDL-C, LDL-C, sUA, BUN, sCr and CgA. (Enter)			
Significance level was set at $P < 0.05$.			
CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; sCr, serum creatinine; BUN, blood urea nitrogen; sUA, serum uric acid; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; CgA, Chromogranin A.			

ROC analysis

ROC analysis was performed to assess the sensitivity and specificity of serum CgA as the potential biomarkers in prediction of early DN. The ROC analysis of serum CgA yielded an AUC of 0.714 (95% CI, 0.639–0.788; $P < 0.001$) in the differentiation of T2DM patients with early DN (Table 4 and Fig. 2). The diagnostic sensitivity and specificity of serum CgA for early DN was 69.86% and 66.12% when the cut off value was 3.46 ng/mL.

Table 4

Validity of serum CgA in prediction of early DN. (in patients with T2DM)

Parameters	CgA
AUC	0.714
95% CI	0.639–0.788
Youden index J	0.360
Cut-off	3.46
Sensitivity (%)	69.86
Specificity (%)	66.12
P-value	< 0.001
CI, confidence interval; AUC, Area Under Curve; CgA, Chromogranin A.	

Discussion

DN is an important microvascular complication of diabetes mellitus. Up to now, there is no satisfactory method to prevent early DN. According to KDOQI definitions [13], DN diagnosis is based on increased albuminuria and decreased eGFR. There are some shortcomings in using albuminuria

as a diagnostic marker for (early) DN. The change of glomerular basement membrane structure may be earlier than the increase of albuminuria [14]. About 30–45% T2DM patients who develop kidney disease associated with decrease in glomerular filtration are observed no increased albuminuria [15, 16]. Meanwhile, albuminuria is not specific for DN. Hypertension or obesity may also affect the filtration barrier of the glomeruli leading to increased albuminuria in patients with T2DM [17]. However, treatment of hypertension usually uses renin angiotensin aldosterone inhibitors, which reduce glomerular hydrostatic pressure and make proteinuria in the normal range. These factors affect the accuracy of diagnosis based on current guidelines. When the kidney of DN is damaged, the decrease of eGFR occurs quite late. The early damage is often accompanied by hyperfiltration [18]. Therefore, both routine markers of DN (albuminuria and eGFR) reflecting glomerular damage has certain limitations.

CgA is the main member of the chromogranins family consisting of water soluble acidic glycoproteins. It was first found in the secretory granules from adrenal medullary chromaffin cells, and released into circulation after splanchnic nerve stimulation together with catecholamines [19]. CgA also exist in a lot of endocrine and neuroendocrine cells [20], nerve cells [21] and immune cells [22]. Elevated serum CgA levels have been found in many cancers [23] and neurodegenerative diseases such as Alzheimer's disease [24] or Parkinson's disease [25]. Previous study has showed that CgA, like many other low MW proteins, is handled by the kidney [26]. However, serum CGA increases in renal failure more than creatinine and the other studied low MW proteins, β_2 -MG(β_2 microglobulin) and TATI (tumor associated trypsin inhibitor) [26]. The study by Chen et al. suggests CgA-stimulation of endothelial cell exocytosis of endothelin as a possible mechanism for regulation of renal function in health and disease [27].

Our study aimed to investigate the role of serum CgA in early diagnosis of DN in patients with T2DM. Because our groups were matched regarding age and sex, the effect of these factors on the results of serum CgA in our study were excluded. In our study we found there was a significant difference among normoalbuminuria, microalbuminuria and macroalbuminuria groups regarding known diabetes duration. This is consistent with other previous studies [28, 29].

In our study, the level of serum CgA in patients with T2DM was much higher than that in the healthy people. Previous study has documented the clinical value of measurements of CgA as a potential marker for diabetes [30]. The concentration of plasma and salivary CgA was significantly higher in diabetic groups compared to the control [31]. Our results are consistent with their findings.

Serum concentrations of CgA correlated strongly with eGFR [32]. Its relationship with GFR is very similar to that of other substances eliminated by the kidney like creatinine and TATI [26]. In our result, Spearman rank correlation test demonstrated that serum CgA was negatively correlated with eGFR, not only in patients with T2DM, but also in all subjects. There was moderate intensity positive correlation between serum CgA levels and Known diabetes duration, UACR in all subjects.

We found for the first time that serum CgA was increasing gradually with the degree of DN, serum CgA level was associated with the occurrence of early DN. One study showed that serum CgA was a poor indicator of DN since patients with T2DM and reduced glomerula filtration rate failed to show any significant increase in serum CgA [33]. In their research, patients were divided into those without DN with normoalbuminuria ($n = 27$), patients with DN with microalbuminuria ($n = 8$), or macroalbuminuria ($n = 42$). It is noteworthy that in their study P value of the difference between patients with normoalbuminuria and DN was 0.07. The failure to reach the statistical difference may be due to the small sample size. Moreover, other studies have found that patients with renal impairment displayed elevated concentrations of CgA in plasma and the CgA concentrations reflect deterioration of renal function [26, 34]. CgA contains multiple amino acid motifs prone to endoproteolytic cleavage resulting in multiple processing fragments. This constitutes a biochemical challenge for accurate quantification of CgA. Several assays for measurements of CgA have been developed, but since the used antibodies detect different epitopes, the results from the assays vary considerably [35]. The assay kits we used were different from those used in mojiminiyi OA's team [33].

Roc analysis showed diagnostic accuracy of serum CgA in differentiation between T2DM patients with early DN and without DN. Using cut-off 3.46 ng/mL for serum CgA, we found sensitivity 69.86%, specificity 66.12%, and diagnostic accuracy 71.4% in predicting early DN.

The findings are limited by the sample size and the crosssectional design of the study therefore this cannot be used to determine the direction of causality sequence. Additional prospective studies are needed to examine associations between CgA with early DN risk.

Conclusion

The results of this study show that serum CgA levels were higher in patients with T2DM compared to healthy subjects, and increased gradually with the degree of DN. It also can be used as a biomarker in early detection of DN.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

HY, HpW, JF and HW participated in the conception and design of the study, and analyzed the data of study. HY drafted the initial manuscript, JF and HW revised the manuscript. XS, AIC, XmY, YmW and BbZ participated in data collection. All authors have read and approved the final manuscript.

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Availability of data and materials

The current data are available to all interested researchers upon reasonable request. Requests for access to data should be made to the principal investigators of the study.

Ethics approval and consent to participate

All subjects have given their written informed consent. The study protocol has been approved by the institutional committee on human research.

Consent for publication

Not applicable.

Competing interests

The authors have no conflicts of interest to declare.

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Figures

Figure 1

Comparison of serum CgA levels in subgroups of subjects divided according to UACR and healthy subjects. (& P < 0.05, && P < 0.001 vs. healthy controls; ** P < 0.001 vs. normoalbuminuria group; # P < 0.05 vs. microalbuminuria group.)

Figure 2

Roc curve for validity of serum CgA in prediction of early DN.