

Human kallikrein 10: a novel tumor marker for ovarian carcinoma?

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Abstract

Background: Human kallikrein 10 (hK10, encoded by KLK10 gene) is a recently discovered member of the human kallikrein family. hK10 is a secreted serine protease. With the development of a highly sensitive and specific immunoassay for hK10, quantification of hK10 in the circulation is now feasible. Our aim was to investigate whether hK10 concentration in serum changes in various malignancies.

Methods: We used a highly specific and sensitive immunofluorometric assay to quantify hK10 protein in 374 serum samples from healthy individuals and patients with various malignancies.

Results: Serum hK10 concentration was found to be significantly elevated in 56% of the ovarian cancer patients and such an increase was not observed in serum of healthy individuals or in serum of patients with other types of cancer, with the exception of ~15% of patients with gastrointestinal cancer. This hK10 elevation does not correlate well with CA 125. We have further demonstrated that hK10 concentration changes during ovarian cancer progression.

Conclusion: This is the first report describing that hK10 serum concentration is significantly elevated in the majority of ovarian cancer patients. Our results indicate that hK10 may be a potential new serological marker for ovarian cancer diagnosis and monitoring. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Human kallikrein 10; Tumor markers; Ovarian cancer biomarkers; Kallikrein gene family; Cancer diagnosis and monitoring; Normal epithelial cell-specific 1 gene (NES1)

1. Introduction

The human tissue kallikrein gene family is a group of genes that are clustered on chromosome

19q13.3–q13.4 and share significant homologies at both the nucleotide and amino acid levels [1–5]. Until recently, this family was thought to contain only three genes, including KLK1 (encoding for human kallikrein 1, hK1; also known as pancreatic/renal kallikrein), KLK2 (encoding for human kallikrein 2, hK2), and KLK3 (encoding for human kallikrein 3 [hK3]; also known as prostate-specific antigen [PSA]). Over the last 2–3 years, new genes were identified in the same chromosomal region and

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are now considered to be members of the kallikrein gene family [6]. These new kallikrein genes are currently known with various empirical names. An international group of investigators has recently agreed on new human kallikrein gene nomenclature [7]. This gene family now contains at least 15 genes which are designated KLK1...KLK15, while their encoded proteins are designated as hK1...hK15.

The normal epithelial cell-specific 1 (NES1) gene is one of these newly identified genes. With the new nomenclature, which will be used exclusively in this paper, NES1 is designated as KLK10 and the encoded protein as hK10. KLK10 was isolated with subtractive hybridization, between radiation-transformed and nontransformed breast epithelial cells [8]. KLK10 resides on chromosome 19q13.4, spans about 5.5 kb of genomic DNA sequence and contains six exons (one untranslated) and five introns [9]. hK10 is a secreted serine protease and its amino acid sequence has 35–40% identity and 50–55% similarity with other members of the human kallikrein gene family, including PSA. The physiological function of hK10 has not as yet been elucidated. Since the KLK10 gene is down-regulated in breast cancer cell lines, it is considered to play a role in the regulation of normal cell growth [8]. Goyal et al. [10] have recently suggested that KLK10 may encode for a tumor suppressor gene. When the KLK10 gene was

transfected into the tumorigenic breast cancer cell line MDA-MB-231, its growth in soft agar was reduced and when this cell line was inoculated into nude mice, tumor formation was significantly decreased.

We have recently developed a new, highly sensitive, and specific immunofluorometric assay for hK10 and demonstrated that this protein is detectable in various biological fluids, including serum, breast milk, amniotic fluid, seminal plasma, and cerebrospinal fluid [11]. The expression of KLK10 appears to be altered in some disease states, including breast and prostate cancer [8,10,12]. Thus, we hypothesized that serum hK10 protein concentration might change during cancer initiation and progression in a manner similar to that observed for other members of this family, including PSA and hK2 [13–16]. In this paper, we report the measurement of hK10 in sera obtained from patients with various malignancies.

2. Materials and methods

2.1. Subjects

The samples used in this study were residual sera of routine testing from patients with various malignancies and from apparently healthy individuals. We

Table 1
Concentration of human kallikrein 10 (hK10) in serum of normal individuals and patients with various malignancies

Patient group	Number of samples	hK10 ($\mu\text{g}/\text{l}$)				Number of patients with hK10 > 1.5 $\mu\text{g}/\text{l}$ (%)	Number of patients with hK10 > 0.8 $\mu\text{g}/\text{l}$ (%)
		Min	Max	Median	95th percentile		
Healthy males	40	0	0.8	0.4	0.7	0 (0)	0 (0)
Healthy females	42	0	0.8	0.3	0.7	0 (0)	0 (0)
Breast cancer ^a	22	0	1.1	0.6	1.0	0 (0)	4 (18)
Medullary thyroid carcinoma ^b	27	0	1.5	0.6	1.4	0 (0)	7 (25.9)
Testicular cancer ^c	51	0.1	1.1	0.6	2.5	1 (2)	6 (11.8)
Gastrointestinal cancer ^d	48	0.1	3.8	0.7	2.2	7 (14.6)	22 (45.8)
Prostate cancer ^e	41	0.2	1.5	0.5	1.3	0 (0)	11 (26.8)
Lung cancer	23	0.2	1.8	0.7	1.6	3 (13)	7 (30.4)
Ovarian cancer ^f	80	0.2	12.2	1.9	9.1	45 (56.2)	62 (77.5)

^aWith serum CA 15.3 levels ≥ 414 kU/l (upper reference range 35 kU/l).

^bWith calcitonin levels ≥ 1135 ng/l (upper reference range 100 ng/l).

^cWith hCG levels ≥ 69 IU/l (upper reference range 10 IU/l) or AFP levels ≥ 110 $\mu\text{g}/\text{l}$ (upper reference range 10 $\mu\text{g}/\text{l}$).

^dWith CA 19.9 levels ≥ 629 kU/l (upper reference range 37 IU/l) and CEA levels ≥ 1000 $\mu\text{g}/\text{l}$ (upper reference range 5 $\mu\text{g}/\text{l}$).

^eWith PSA levels ≥ 1000 $\mu\text{g}/\text{l}$ (upper reference range 4 $\mu\text{g}/\text{l}$).

^fWith CA 125 levels ≥ 372 kU/l (upper reference range 35 kU/l).

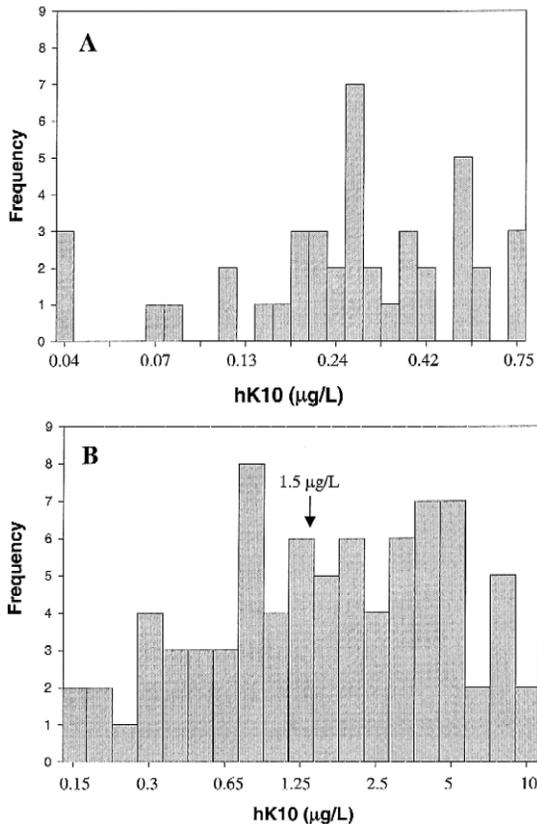


Fig. 1. Frequency distribution of hK10 concentrations in the serum of 42 healthy females (A) and 80 patients with ovarian cancer (B). The level of 1.5 µg/l, used as a cutoff in Table 1, is indicated by an arrow. About 56% of ovarian cancer patients have a serum hK10 concentration higher than this cutoff value.

tested a total of 374 sera from an equal number of patients as follows: 40 healthy men, 42 healthy women, 40 women with breast cancer, 27 patients with medullary thyroid carcinoma (14 men and 13 women), 51 men with testicular cancer, 48 patients with colorectal cancer (25 women and 23 men), 41 men with prostate cancer, 23 patients with lung cancer (10 women and 13 men), and 80 women with ovarian cancer. Disease classification in these patients was based on ICD-9 code from medical chart review (i.e., lung cancer) and/or using appropriate tumor marker levels: CA 15.3 (breast cancer), calcitonin (medullary thyroid cancer), hCG (testicular cancer), CEA and CA 19-9 (colorectal cancer), total PSA (prostate cancer), and CA 125 (ovarian cancer). In order to increase the possibility of detecting hK10

alterations in serum, patients included in this study had relatively high tumor burden (as indicated by a tumor marker level at least 10-fold higher than the upper limit of normal). Since this is a preliminary study, no other criteria were used for patient selection. All serum samples were stored at -20°C until analysis for a maximum time of 1 year. Our procedures are in accordance with the Ethical Standards of the Helsinki Declaration of 1975, as revised in 1983 and have been approved by the Institutional Review Boards.

2.2. Analysis of tumor markers

The tumor marker markers CA125, PSA, CEA, and AFP were analyzed on the Elecsys immunoassay analyzer (Roche Diagnostics, Indianapolis, IN). CA 15.3, CA 19.9, and hCG were analyzed with the Immuno 1 immunoassay analyzer (Bayer Diagnostics, Tarrytown, NY) and calcitonin was measured with a radioimmunoassay kit from Diasorin (Italy). The upper limit of normal for these tumor markers is 35 kU/l (CA 125), 4 µg/l (PSA), 10 µg/l (AFP), 5 µg/l (CEA), 35 kU/l (CA 15.3), 37 kU/l (CA 19.9), 10 IU/l (hCG), and 100 ng/l (calcitonin).

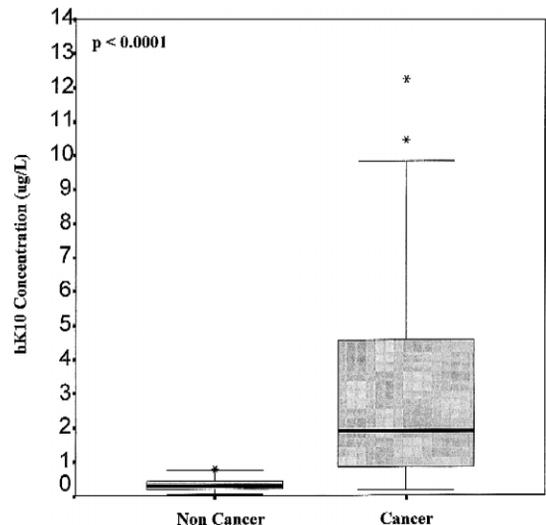


Fig. 2. Distributions of hK10 concentrations in ovarian cancer and noncancer females. The boxplots display the quartiles. Asterisks indicate the outliers. The p value was determined by Mann-Whitney U -test. Thick horizontal lines show medians.

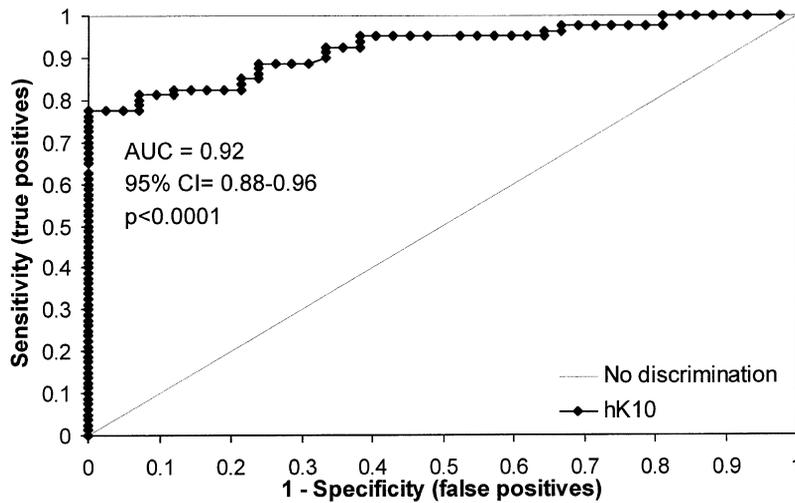


Fig. 3. Receiver operating characteristic (ROC) curves for hK10 serum concentration, demonstrating the potential of the variable in the ovarian cancer diagnosis (CI, confidence interval; AUC, the area under the ROC curve).

2.3. Immunofluorometric assay for hK10

Our hK10 immunoassay utilizes two hK10-specific polyclonal antibodies, one raised in mice and the other in rabbits. The details of this assay have been recently described [11]. In brief, mouse anti-hK10 antiserum was captured with sheep anti-mouse IgG antibody (Jackson ImmunoResearch, West Grove, PA) immobilized on 96-well white polystyrene microtiter plates. Serum samples (without pretreatment, 50 μ l) were then added into each well followed by incubation and washing. Rabbit anti-hK10 antiserum was subsequently applied, incubated, and washed. Finally, alkaline phosphatase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch) was added, incubated, and washed. To detect the signal, time-resolved fluorometry was used, essentially as described elsewhere [17]. The measurement range of this assay is 0.05–20 μ g/l with a precision of less than 10% across the measurement range.

2.4. Statistical analysis

Relationships between different variables were assessed by Pearson correlation coefficient. Medians were compared by the nonparametric Mann–Whitney test. Receiver operating characteristic (ROC) curves were constructed by plotting sensitivity vs. (1 – specificity) and the areas under the ROC curves

(AUCs) were calculated. For all analyses, $p < 0.05$ was considered statistically significant. SAS software (SAS Institute, Cary, NC) was used for statistical analysis.

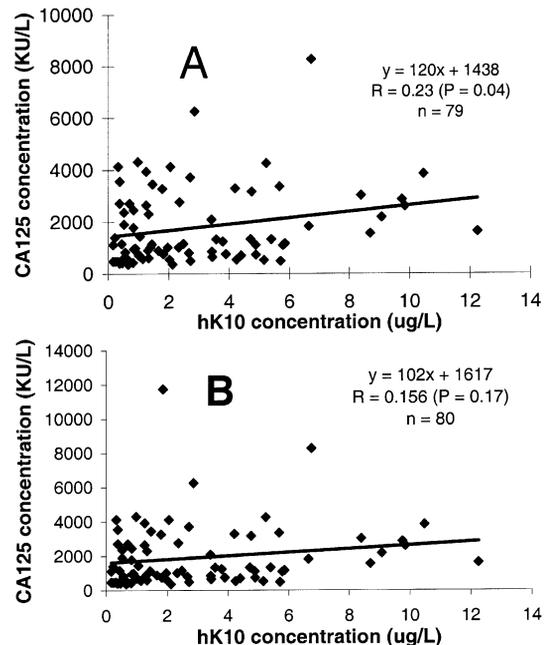


Fig. 4. Correlation between CA125 and hK10 concentration in 79 serum samples from ovarian cancer patients (A) and in 80 serum samples (B). One value was excluded from analysis in panel (A).

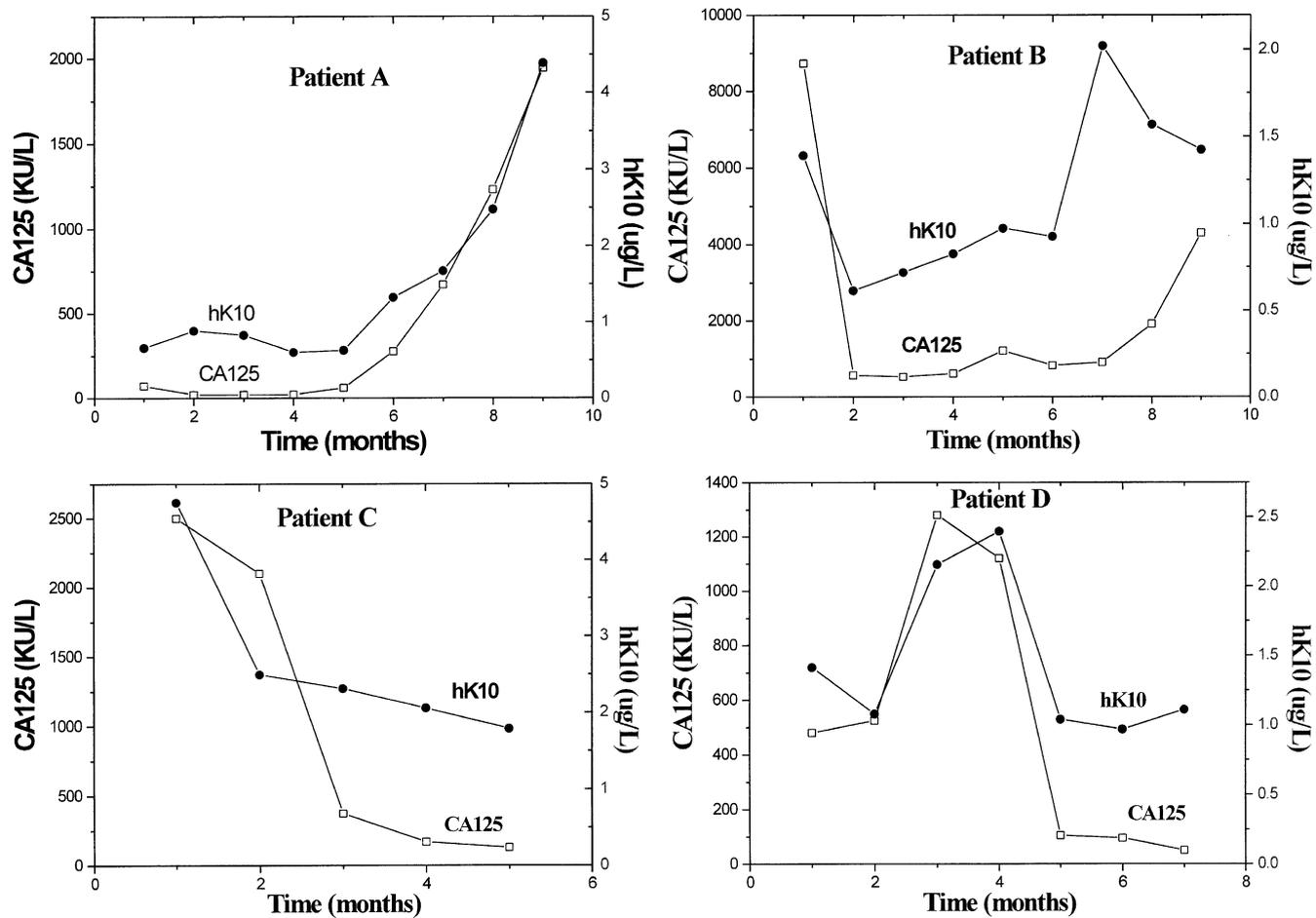


Fig. 5. Analysis of hK10 and CA 125 in serial serum samples from ovarian cancer patients, who were monitored postprimary treatment. For more discussion, see text.

3. Results

We analyzed a total of 374 serum samples from either individuals without known malignancies or patients with various malignancies, with a recently described sensitive and specific hK10 immunoassay [11]. The results are shown in Table 1. The majority (56%) of the ovarian cancer patients had serum hK10 levels higher than twice the upper limit of healthy subjects ($1.5 \mu\text{g/l}$). However, none of the healthy controls, breast cancer, medullary thyroid carcinoma, and prostate cancer patients had serum hK10 concentration higher than this cutoff value. The other malignancies screened only had a small proportion of patients with serum hK10 levels higher than this cutoff, including gastrointestinal cancer (15%), lung cancer (13%), and testicular cancer (2%). When we chose the value of $0.8 \mu\text{g/l}$ (the 100th percentile value for healthy males and females as cutoff), the positivity rate for ovarian cancer increased to 78%, however, a significant number of patients with other malignancies were also positive (Table 1). The distribution of hK10 in the serum of healthy female and ovarian cancer patients is shown in Fig. 1, and as a boxplot, is shown in Fig. 2. The medians between the two groups are significantly different ($p < 0.0001$ by Mann–Whitney test). ROC curve analysis is further shown in Fig. 3. hK10 (AUC, 0.92; 95% confidence intervals [CI], 0.88–0.96) was a strong predictor of ovarian cancer. At 95% sensitivity and 36% specificity, the hK10 serum concentration was $0.230 \mu\text{g/l}$ while at 95% specificity and 78% sensitivity, the hK10 serum concentration was $0.71 \mu\text{g/l}$.

To investigate the correlation between hK10 and CA 125 in the serum of ovarian cancer patients, least-squares linear regression was performed. As Fig. 4 shows, there is a weak correlation between serum hK10 and CA 125 in ovarian cancer patients ($r = 0.23$, $p = 0.04$, when one value was excluded from the analysis).

To further investigate whether hK10 serum concentration has any value in monitoring disease progression, serial serum samples from four ovarian cancer patients were also analyzed, along with CA 125 values. As shown in Fig. 5, serum hK10 concentration changes in parallel to CA 125 in most cases.

These data suggest that hK10 may have potential for ovarian cancer monitoring after primary treatment.

4. Discussion

Human tissue kallikreins are a group of secreted serine proteases and at least 15 members are currently known [6]. Among all these kallikreins, PSA has been proven to be the most valuable biomarker for prostate cancer [13–15]. hK2 is also a potential new prostatic biomarker, but it is still under investigation [5,16]. The value of the newly discovered kallikreins as biomarkers has not been investigated due to the lack of suitable methods for measuring them in the circulation. Recently, we developed a highly sensitive and specific immunoassay for the measurement of serum concentrations of hK10 [11]. Therefore, the investigation of hK10 as a potential marker for various diseases became feasible.

In this study, we analyzed 374 serum samples from healthy individuals ($N = 82$) and patients with various malignancies ($N = 292$). We found that serum hK10 concentration was significantly elevated ($\geq 2 \times$ the upper reference range) in the majority of ovarian cancer patients, but not in serum of healthy individuals or patients with other types of cancer (Table 1 and Figs. 1 and 2). In serum samples ($N = 79$) from our patients with ovarian cancer, hK10 concentration correlated weakly ($p = 0.04$) with CA 125 concentration (Fig. 4). Also, hK10 serum concentration fluctuates according to the tumor burden, as assessed by analysis of CA 125 (Fig. 5). These results indicate that hK10 may be a new marker for ovarian cancer.

Ovarian cancer is one of the leading causes of death in women [18]. Survival can be dramatically increased if the disease is diagnosed at an early stage [19]. However, early detection is hampered by the lack of a highly sensitive and specific biomarker. Currently, CA 125 is one widely used serum marker for ovarian cancer, but it is not specific or sensitive enough for diagnosis [20–22]. Other newly introduced serum markers [23], such as inhibin [24,25] and OVX1 [26], have shown some promise but have not gained wide acceptance.

Our results suggest that hK10 may have value as a biomarker of ovarian cancer. hK10 was elevated in

a majority of ovarian cancer patients. The specificity can be manipulated by selecting an appropriate cut-off, as shown in Table 1 and in the ROC curve of Fig. 3. However, we also found positive samples from patients with other malignancies, including those from gastrointestinal, lung, prostate, thyroid, testicular, and breast cancer. This is common for many other cancer biomarkers [22]. hK10 does not correlate well with CA 125, which suggests that it may be used in conjunction with CA 125 to achieve more sensitive and specific ovarian cancer detection and monitoring. Further, hK10 changes with tumor burden, suggesting that it may also be useful for disease monitoring (Fig. 5). However, whether hK10 is sensitive enough for early ovarian cancer detection needs further investigation. The samples used in this study have relatively high CA 125 values (at least 10 times higher than the upper reference value). It will be worthwhile to analyze presurgical serum samples from ovarian cancer patients at different stages to examine whether hK10 increases early during ovarian cancer development. Furthermore, analysis of samples from patients with nonmalignant abdominal pathologies, which usually cause false elevations of CA 125 concentration (e.g., endometriosis, ovarian cysts, etc.), will further establish the specificity of the new test.

In conclusion, we present the first evidence that serum hK10 concentration is significantly increased in the majority of ovarian cancer patients and in a smaller proportion of patients with other types of cancer. We suggest that hK10 may constitute a new and useful biomarker for ovarian cancer diagnosis and monitoring. These data need confirmation with a larger group of patients and with prospective clinical studies.

Acknowledgements

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