

ALU repeat as potential molecular marker in the detection and prognosis of different cancer types: A systematic review

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Abstract. Cancer is a major health issue worldwide. cfDNA integrity has been reported as a potential diagnostic molecular marker for different types of cancer, identifying the importance of liquid biopsy. The aim of this review was to evaluate the prognostic and diagnostic performance of *Arthrobacter luteus* (ALU) repeat in tumor. Following a thorough review of the literature published from January, 2000 to September 2021, 36 studies were included. All of the study descriptions were analyzed. According to several studies, there were increased concentrations of ALU repetitive elements in cancer patients, while these concentrations were decreased in control, benign, different cancer stage, and other diseases. The total ALU (115 and 247) sequence levels are potential biomarkers for the purpose of investigations and cancer prognosis.

Introduction

In both developed and developing countries, cancer is a major health issue and a leading cause of mortality that is still on the increase, worldwide. According to the International Agency for Research on Cancer, in 2018, 9.6 million individuals died from cancer, an increase from 8.2 million in 2012 and 7.6 million in 2008 (1-3). Tumorigenesis is a multi-step, multi-factorial disease described by genetic and epigenetic changes, which is difficult to control and prevent (4). In 2012, the WHO's International Agency for Research on Cancer predicted that by 2030, worldwide, there would be 21.7 million newly diagnosed cancer cases and 13 million cancer deaths as a result of population growth and the increase in life expectancy (5).

Cancer is a major health problem that affects individuals globally. Several types of cancer can be avoided if diagnosed

early enough. However, tumors such as lung, colon, and breast cancers frequently have late-stage diagnosis. Despite efforts to ensure survival is prolonged, only a moderate improvement has been achieved in cancer patients. Failure to diagnose cancer early generally leads to ineffective treatment and an even worse prognosis. The availability of robust diagnostic biomarkers is critical for diagnosing cancer patients at an early stage and thereby greatly reducing overall mortality rates. (6).

Circulating molecular biomarkers have increasingly been used as a liquid biopsy in the peripheral blood and have the benefit of being easily accessible, with early detection, and reproducibility (7). Circulating tumor cells, circulating DNA, and microRNAs have been studied as a detection tool and prognosis of various cancer types (8-13).

DNA is a molecule that may be found inside and outside of cells. Extracellular DNA can be found in blood and other body fluids. Cell-free DNA refers to the degraded DNA fragments floating in the circulation (cfDNA). DNA in the bloodstream releases apoptotic or necrotic cells. The length of DNA fragments and distribution of DNA size could signify cfDNA source (14). Apoptosis of the cell naturally occurs, and DNA is divided into similar fragments of 185-200 bp. However, tumor necrosis produces similar fragments of DNA in variable lengths generally >200 bp (15). Circulating tumor DNAs (ctDNAs) based DNA integrity index served as a possible indicator of prognosis in hepatocellular carcinoma, lymphoma, colorectal, lung, and breast cancer (15-19). DNA analysis can be conducted on the basis of ctDNA (from a liquid biopsy) as well as directly isolated DNA from tumor tissue acquired by biopsy or excision (20).

According to Iqbal *et al* (21) presence of cfDNA in blood, although reported in 1948 by Mandel and Metais (22), was rediscovered after 30 years in autoimmune disorders by Tan *et al* in 1966 (23) and in cancer by Leon *et al* in 1977 (24).

Apoptosis is the source of cfDNA in a healthy person, raising shorter and evenly sized DNA fragments. Furthermore, in cancer, necrosis results in unequal longer DNA fragments in addition to the shorter apoptotic fragment (25-27). As a result, higher levels of longer DNA fragments in the blood have been identified as a useful indicator of the existence of malignant tumor DNA (26-28). The cfDNA concentration in serum is higher in patients with cancer when compared to healthy individuals (5,29-31).

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The variability of cfDNA levels in patients is most probably associated with tumor stage, burden, cellular turnover, vascularity, and response to therapy with the highest levels reported in patients with metastatic and advanced disease (32).

cfDNA levels have been found to be elevated in a variety of cancers (7). One measure of cfDNA fragmentation is cfDNA integrity (cfDI), which is calculated as the ratio of longer to shorter DNA fragment concentrations at the same genetic location (29).

A liquid biopsy is a viable alternative consisting of the circulating analysis (cfDI). This main advantage of this method is that it is less invasive, using just a sample of peripheral blood. During the past two decades, cfDI analysis has emerged as a promising tool for cancer diagnosis and prognosis (33,34).

The *Althrobacter luteus* (ALU) repeats are the most predominant repetitive sequences in the human genome, 300 bp in length, with 1.4×10^6 copy number per genome. Most studies used DNA integrity, defined as the ratio of ALU 247 long fragments released from necrotic cells and ALU 115 short fragments released from normal cells (35).

ALU-quantitative PCR (qPCR) has become the most widely used technology for detecting the DNA integrity index (32). ALU covers over 10% of the human genome (36). Research has been conducted to assess the potential use of cfDI from the ALU variable as a diagnostic biomarker for a variety of cancers, such as breast and prostate cancer (37).

A higher portion of longer DNA fragments has been recommended as a cancer detection biomarker (26). Several formulae have been presented to objectively calculate the 'DNA integrity index' as a ratio of longer and smaller fragments. Umetani *et al* (27,38) determined the pure ratio of ALU 247 and ALU 115 concentrations in patients' blood, while Wang *et al* (39) assessed DNA integrity in patient plasma using a calculation based on delta-Cp values. Patients with ovarian, breast and colorectal cancer had higher DNA integrities in serum and plasma than controls, according to both authors (27,38). Other studies, on the other hand, could not find a difference in DNA integrity values in the same tumor types (40-42).

However, in different studies, the performance of ALU repeat as a biomarker for cancer diagnosis varied widely. Therefore, this systematic study, to the best of our knowledge, is the first to clarify the diagnostic and prognostic role of ALU elements as a molecular marker of cancer.

Materials and methods

Strategy of search and study selection. A search for potentially suitable articles was performed on the PubMed online databases up to September 2021, for research articles. The following keyword combinations were included in the detailed search strategy: ('ALU' OR 'cfDNA') AND ('cancer' OR 'tumor'). Studies were considered for selection if they included information on ALU sequences and their potential role in the diagnosis or prognosis of different types of cancer. Studies not in English or where only the abstract was available were excluded. Initially, data extraction was carried out by two of the authors (AS and SS). The full-text articles were then obtained for more evaluation. The reference lists of all of the studies were manually checked by the authors to identify additional publications that may be of interest.

The studies that were determined to be eligible were as follows: a plan for an observational, assessing the relationship between ALU and the role in diagnosis or prognosis of cancer, there was enough information to assess the difference in ALU levels between the patients and the controls and between cancer stage.

Collection of data and quality assessment. Authors extracted data independently from each eligible study and abstracted the following information including cancer type, sample type, DNA size ratio. The data were evaluated for each group, and the main results identified.

Included studies. The PubMed search identified 250 articles. After the initial inclusion of 150 articles (based on title and abstract were selected for assessment), 32 articles were excluded due to not meeting the criteria for inclusion (including ALU methylation, comparison between ALU and LINE, and studies in other languages). A manual search of the references of studies on the subject yielded 15 additional articles. A total of 133 articles were included for the full text assessment, in English. The selection of the study flowchart is presented in Fig. 1.

Results

Included studies. The PubMed search initially identified 250 articles. Based on title and abstract 150 articles were selected for assessment, of which 32 articles were excluded due to not meeting the criteria for inclusion. Further manual search of the references yielded 15 additional articles. A total of 133 articles were included for the full text assessment (Fig. 1).

Overall characteristics. The characteristics of the studies that were included are shown in Table I, which shows the number of tumor cases (16-268) and healthy controls (12-110). The subjects' age range was 18-71 years. There were 16 studies from European countries (44.4%), 12 from Asia (33.3%), 6 from Africa (16.7%), and 2 from the USA (5.6%). These studies focused on carcinoma, including breast, lung, prostate, ovarian, endometrial, pancreatic, and thyroid cancer. To determine the value of cfDI, all of the included studies used the quantitative PCR (qPCR) method, 14 of them were evaluated in serum and 19 in plasma, 1 (in serum and plasma), 1 in tissue, and 1 in urine. cfDI was calculated as the ratio of longer DNA fragment concentrations to shorter ones in the same locus. The reference list included 35 articles, published between 2006 and 2021.

ALU as diagnostic or prognostic biomarker in cancer. When comparing cancer cases with control in 36 articles, in 12 studies, the levels of ALU 247 and ALU 115 were higher in patients than in controls. Thus, in total, 11 studies were retained, with cfDI higher in cancer patients than the healthy controls (an association between a higher cfDI and tumor stage, as well as high sensitivity was identified in 3 of 12 articles) and 4 had no cfDI difference.

Table I shows 11 studies had only diagnostic and 8 had only prognostic information, and 5 articles from the cancer group had significantly higher concentrations of ALU sequences and cfDI than the benign disease group. Six studies monitoring the

Table I. List of studies evaluating ALU in cancer.

Cancer type	No. of subjects	Source of DNA	Ratio of DNA size	Conclusions	(Refs.)
Breast cancer	(51) Controls (83) Preoperative (Stage 0 to IV primary BC)	Serum	ALU 247/115	Serum circulating cfDI is a potential molecular biomarker for detecting the progression of BC and lymph node metastases	(27)
Breast cancer	(49) Controls (39) BC patients	Plasma	ALU 247/115	According to the results, necrosis could be a possible source of cfDNA ALU 247; and a tumor biology phenotypic feature	(43)
Breast cancer	(65) BC patients undergoing neoadjuvant chemotherapy No controls reported	Plasma	ALU 247/115	The study indicates circulating DNA biomarkers ALU (115 and 247) as two potential future markers for the assessment of neoadjuvant chemotherapy response in BC patients	(44)
Breast cancer	(28) Controls (12) Benign BC patients, (65) Locally confined BC patients (47) Metastatic breast cancer patients	Plasma	ALU 247/115	Plasma DNA is helpful in the diagnosis of locally BC, however, in MBC, established tumor markers are the most informative	(45)
Breast cancer	(100) Controls (82) Primary BC patients (201) MBC patients	Plasma	ALU 260/111	Study shows that cfDI was reduced and cfDNA level increased can be used as diagnostic biomarkers for both primary and metastatic breast cancer, and cfDI as an MBC prognostic marker, as a result, they're good candidates for blood-based multi-marker tests	(10)
Breast cancer	(51) Controls (148) BC patients (148) Baseline (47) Postoperative	Serum	ALU 247/115	In patients with primary BC, cfDNA level and cfDI were found to represent potential prognostic markers	(21)
Breast cancer	(175) Non-recurrent BC patients (7) recurrent-BC patients No controls reported	Plasma	ALU 260/111	In the clinic, cfDI could be a helpful biomarker for prognosis of BC recurrence when combined with other molecular markers	(46)
Breast cancer	(268) MBC patients No controls reported	Plasma	ALU 260/111	At baseline and during systematic therapy, cfDNA variables can serve as attractive prognostic markers for MBC patients, especially when combined with other markers	(18)
Breast cancer	(10) Controls (40) BC patients (2: stage I, 31: stage II, 2: stage III, and 5: stage IV)	Plasma	ALU 247/115	Both ALU 247 and ALU 115 appear to be prognostic markers for BC preoperative	(47)
Breast and prostate cancers	(64) Females (Consisting of 32 controls 32 and BC patients) and (61) Males (Consisting of 30 controls and 31 prostate cancer patients)	Serum	ALU 247/115	cfDI increased with disease severity and higher staging in the prostate but not in BC	(5)
Breast and lung cancers	(64) Controls (64) BC patients (64) Lung cancer patients	Plasma	ALU 263/58	This study suggested ALU index could be used as a test to discriminate cancer patients from healthy individuals	(34)

Table I. Continued.

Cancer type	No. of subjects	Source of DNA	Ratio of DNA size	Conclusions	(Refs.)
Prostate cancer	(96) PC patients (112) Benign prostate hyperplasia	Plasma	ALU 247/115	cfDNA and cfDI could be used to differentiate PC from BPH in patients with serum PSA C 4 ng/ml	(48)
Prostate cancer	(30) Controls (50) PC patients (25) BPH	Serum	ALU 247/115	ALU 115 could be a useful biomarker for identifying patients that are at high risk, pointing to early tumor cell spread as a possible seed for future metastases	(49)
Prostate cancer	(30) Controls (30) PC patients (40) BPH	Plasma	ALU 247/115	A significant relationship between cfDNA concentration, its integrity, and PC suggests that the liquid biopsy can be used as a non-invasive early diagnostic biomarker	(50)
Ovarian cancer	(12) Controls (24) Ovarian cancer patients (12) Benign ovarian cysts patients	Plasma	ALU 219/115	Monitoring ALU concentrations, alone or in combination with other tumor markers, could be used for subsidiary diagnosis and prognosis of ovarian cancer	(51)
Ovarian cancer	(28) Controls (37) Ovarian cancer patients	Plasma	ALU 260/111	In combination with other molecular markers, cfDNA variables could be used as diagnostic biomarkers in ovarian cancer	(29)
Endometrial cancer	(15) Controls (53) EC patients (9) Benign gynecologic disease patients	Plasma	-	Although cfDNA measurement is not effective for EC screening, the change in cfDNA in a patient could be a prognostic biomarker for EC	(52)
Endometrial cancer	(60) Controls and EC patients	Serum	ALU 247/115	The study noted the potential use of serum cfDI as a noninvasive molecular biomarker in EC. And a correlation analysis between cfDNA quantitative and qualitative content and clinicopathologic characteristics, such as body mass index, blood pressure level, and lymphovascular space invasion status	(32)
Endometrial cancer	(32) EC patients No controls reported	Plasma	ALU 247/115	Decreased plasma cfDI during vaccination and the cfDI was related to prognosis. Another cancer study has confirmed some of these findings, as a result, the cfDI could be a potential biomarker for future cancer vaccination therapies	(53)
Pancreatic malignancies	(23) Controls (50) Pancreatic ductal adenocarcinoma patients (23) Pancreatic neuroendocrine tumor patients (20) Chronic pancreatitis patients	Plasma	ALU 244/83	The lack of detectable cfDNA levels in pancreatic diseases has a significant impact on the clinical usage of such a biomarker in pancreatic ductal adenocarcinoma patients. When evaluating the diagnostic value of cfDNA in pancreas pathology, different methods of analysis should be used	(54)
Pancreatic cancer	(19) Controls (19) Pancreatic cancer patients	Serum	ALU 247/115	cfDI is not a useful biomarker to detect premalignant pancreatic tumors	(55)
Pancreatic cancer	(32) Control adjacent pancreatic tissue specimens (42) The tumors samples	Tissue	ALU 247/115	cfDI (ALU 247/115 ratio) was no significant difference between pancreatic cancer patients and controls	(14)
Colorectal cancer and periampullary cancer	(51) Controls (32) CRC patients (19) Periampullary cancer patients	Serum	ALU 247/115	cfDI is a promising serum biomarker for colorectal and periampullary cancer detection and evaluation	(38)

Table I. Continued.

Cancer type	No. of subjects	Source of DNA	Ratio of DNA size	Conclusions	(Refs.)
Colorectal cancer	(35) Patients without endoscopic abnormality (26) Benign colorectal adenomas patients (24) CRC patients	Serum and plasma	ALU 247/115	In patients with positive fecal occult blood tests, the circulating marker, in combination with other markers, offers the possibility of a simple blood test as a secondary screen for CRC and polyps	(56)
Colorectal cancer	(24) Controls (24) CRC patients (11) Benign gastrointestinal diseases patients	Serum	ALU 247/115	cfDI is significantly higher in CRC patients and could be useful in future studies	(57)
Colorectal cancer	(110) Controls (104) Primary CRC patients (85) Operated colorectal cancer patients (16) Recurrent/metastatic CRC patients (63) Intestinal polyps' patients	Serum	ALU 247/115	Combined with ALU 115, the ratio of ALU 247/115 and carcinoembryonic antigen detection could enhance CRC diagnostic efficiency. Serum cfDNA and cfDI may be valuable in early diagnosis and monitoring of CRC progression and prognosis	(30)
Colorectal cancer	(20) Controls (50) CRC patients (10) Benign colonic polyp's patients	Serum	ALU 247/115	As a potential serum biomarker, the cfDI outperforms the absolute DNA level for CRC diagnosis. It could also be used as a marker for monitoring the progression of CRC patients	(58)
Colorectal cancer	(56) Controls (114) CRC patients (22) Adenomatous lesion patients	Serum	ALU 244/83	Serum cfDNA concentrations may be an effective source of non-invasive cancer biomarkers	(59)
Colorectal cancer	(30) Controls (90) CRC patients (30) Benign colorectal mass patients	Serum	ALU 247/115	According to the study, cfDI is better to carcinoembryonic antigen as an early biomarker for detecting CRC and its potential to be employed as a biomarker for malignancy	(35)
Colorectal cancer	(76) Primary CRC patients who underwent surgery, including (60) with chemotherapy and (43) with follow-up No controls reported	Serum	ALU 247/115	Serum cfDI may be a promising candidate biomarker for prognostic prediction in CRC patients who have had chemotherapy and are being followed-up for a short time	(60)
Thyroid cancer	(29) Benign nodules patients (38) Malignant lesions patients No controls reported	Plasma	ALU 247/115	Measured the integrity index in the vein draining the thyroid is similar to that measured in the antecubital vein, using a peripheral liquid biopsy to validate cfDI measurements. In opposition to its diagnostic efficacy in aggressive cancers, cfDI has limited utility as a biomarker of malignancy in cytologically indeterminate thyroid nodules	(33)
Non-small cell lung cancer	(40) Controls (60) Non-small cell lung cancer patients (40) Chronic obstructive pulmonary disease patients	Serum	ALU 247/115	Serum cfDNA level, its integrity may be an effective tool of NSCLC early diagnosis and prognosis of the disease	(61)
Non-small cell lung cancer	(107) Controls (106) NSCLC patients (105) Tuberculosis patients	Plasma	ALU 247/115	NSCLC may be identified from tuberculosis with cfDNA and cfDI as indicators. Furthermore, the integrity index had a significant effect on traditional tumor markers in distinguishing NSCLC from tuberculosis	(31)

Table I. Continued.

Cancer type	No. of subjects	Source of DNA	Ratio of DNA size	Conclusions	(Refs.)
Non-small cell lung cancer	(130) NSCLC patients	Plasma	ALU 247/115	The findings show that cfDI could be used as a prognostic biomarker in patients who received a personalized peptide vaccine	(62)
Lung cancer	(19) Controls (29) Lung cancer patients	Plasma	ALU 247/115	The study suggests that ALU repeat ratios could be used for prognostic purposes in the advanced setting for patients of lung cancer patients	(15)
Lung cancer	(35) Controls (55) Lung cancer patients	Urine	ALU-60, 115 and 247	cfDNA concentration index could serve as promising diagnostic biomarkers for lung cancer	(63)

ALU, *Arthrobacter luteus*; BC, breast cancer; MBC, metastatic breast cancer; PC, prostate cancer; BPH, benign prostate hyperplasia; EC, endometrial cancer; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; cfDNA, cell-free DNA; cfDI, circulating-free DNA integrity.

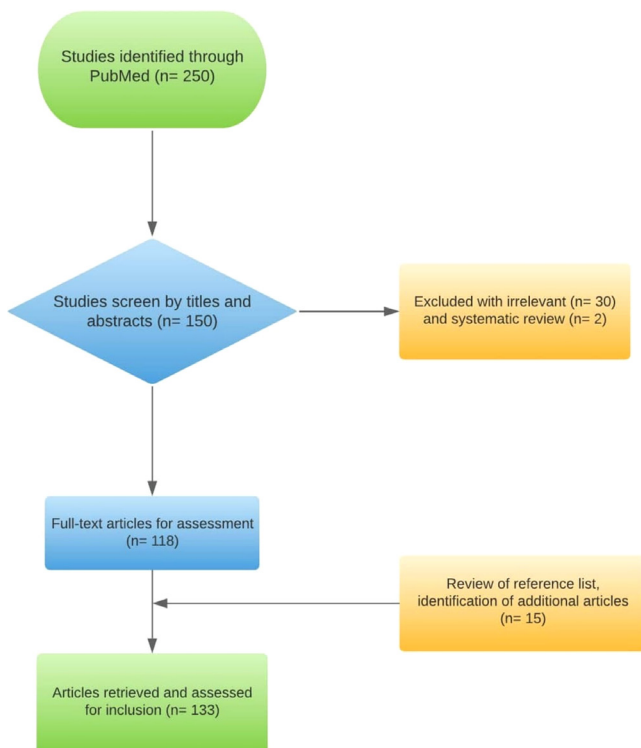


Figure 1. Flowchart of the present study showing how the 133 articles were selected.

ALU level could be applied for subsidiary cancer diagnosis, either alone or in combination with additional tumor markers.

For other ALU, including ALU 260/111, the cfDNA variables can serve as attractive prognostic markers for metastatic cancer during therapy. In addition, although ALU 244/83 is a potential biomarker, there are currently no extensive studies to verify this hypothesis (Table I).

Discussion

Cancer is a major global health problem due to the increasing incidence and fatality rates. An early cancer diagnosis is

crucial as it can improve the chances of survival for cancer patients and decrease the mortality rate. At present, liquid biopsies are promising due to their potential advantages, including reliability, easy access, and reproducibility (11).

In the present review, several studies supported the use of liquid biopsy in cancer as being innovative. To determine whether this liquid biopsy could assist the diagnostic or assessment of treatment response, 36 articles were included to identify the ALU sequences as a biomarker in cancer. The limitations of data retrieved are mostly related to the 36 articles as there is great heterogeneity in studies, it is difficult to analyse the subgroup study, the articles constitute a small sample size, little research highlighting the differences of ALU and cfDI at different stages is available, and the cut-off values vary widely between studies and were missing in other studies.

The present review identified the role of ALU element in cancer progression. Collectively, our data indicated that ALU elements can be used as a biomarker (29,35,44,47,49,52,54,63). The use of cfDNA, for early diagnosis, prognosis biomarkers and monitoring of therapy have been a significant advancement in clinical medicine (18,47,51,52,60-62).

As mentioned previously, not all studies have confirmed that ALU levels vary with tumor development and progression, which may elucidate that cancer is a heterogeneous disease.

cfDI was subsequently evaluated for its usefulness in cancer diagnosis and prognosis (5,15,31,32,34,48,58). Higher cfDI values in cancer patients vs. healthy controls were identified in many studies (31,34,57,58,61). By contrast, lower cfDI was observed in different studies; however, some articles with a focus on metastatic breast cancer (10,45), recurrent breast cancer (46), or first cycle of vaccination (53,62) were few and inconsistent.

There are few studies highlight of the ALU 260/111 in cancer (10,18,29,46) so is not possible to determinate the role of it as biomarker.

Several studies have identified an altered cfDI in patients compared to controls. However, these studies are heterogeneous, some studies showed a reduced cfDI in patients, while others reported an increased cfDI. Various hypotheses have been posited to understand the underlying reason.

cfDNA from healthy individuals had 3- to 5-fold multiples of nucleosome-associated DNA length, and longer fragments than cfDNA from pancreatic cancer patients using direct visualization by gel electrophoresis (64). Jiang *et al* (64) found increased levels of shorter mitochondrial DNA molecules in the plasma of cancer patients compared to healthy people using massively parallel sequencing.

The present study included 36 studies with many discrepancies. This was due to the fact that they were highly heterogeneous studies and not all studies included different stages. In addition, not enough data were available for a specific cancer type and occasionally subjects were limited, there were differences in molecular methods used and source of samples could influence the results. This heterogeneity may affect clarification and whether ALU can act as a biomarker in cancer disease. Thus, further studies are needed to better clarify the role of the ALU element in specific subgroups.

In general, the results obtained from the present systematic review show that the expression of ALU-247 and ALU-115, and cfDI concentration is a rising trend associated with cancer and cancer stage. As a result, circulating cfDNA may be significantly related to tumor cell turnover and tumor progression, indicating biologic tumor aggressiveness. Thus, the circulating cfDI could be suitable for monitoring cancer progression. In addition, large and multicenter sample groups must be studied to corroborate the findings.

Liquid biopsy is becoming the focus of future tumor diagnosis and treatment research. It is less invasive and painful, can be collected in a short period of time, can be collected regardless of where the target organ is located, can be repeated continually, and can represent cancer volume in real-time. However, there are disadvantages, such as if the amount of cfDNA is insufficient, correct information may not be collected. Thus, analytical technology must be established, and the patient's condition or underlying disease may influence the results. Liquid biopsies, as with any other test method, involve large cohort studies to determine their efficacy and sensitivity to various disorders.

The limitation of the present study was mainly to mention the results of ALU and cfDI in blood samples without mentioning the *in vitro* information regarding ALU and cfDI. This aspect should be investigated in future work.

In summary, findings of the review suggest that cfDI can be a significant predictor of developing cancer in patients and could be a useful marker in a molecular, blood-based multi-marker assay.

The current systematic review assessed the total ALU sequence levels and its index are promising biomarkers for the purpose of investigation or prognosis of cancer. However, because of the heterogeneity between studies, the difference of ALU value in different cancers (type or stage) therefore makes it difficult to compare between different types of cancer. Further studies and meta-analyses are needed for the final conclusion to explore the diagnostic role of ALU in malignant diseases, especially when combined with other cancer biomarkers.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

SAS, AMA-R and AAS conceived and planned the review. Data extraction was carried out by SAS and AAS. SAS, AMA-R and AAS evaluated the articles, manually checked references and wrote the review. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable for systematic reviews.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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