Circulating cervical cancer biomarkers potentially useful in medical attention (Review)

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Abstract. Cervical cancer (CC) is a public health problem worldwide, including Mexico. This type of cancer is the fourth most frequent in women worldwide; in Mexico it is the second most common type in women after breast cancer. The diagnosis of CC is based mainly on Pap smears and colposcopy and the identification of molecular tools that serve as a support for these methods is urgent. Regarding this, differential expressions of specific circulating biomolecules has been detected and, based on this, they have been postulated as potential biomarkers for CC diagnosis, prognosis, and/or to identify the response to treatments. Importantly, the combined analysis of these molecules considerably improves their efficacy as biomarkers and their potential use in the medical attention is promising.

Contents

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1. Introduction

Cervical cancer (CC) is the four most common cancer in women with an estimate of 604,000 new cases and 342,000 deaths per year worldwide (1). Most of the new cases (85%) and deaths (90%) occur in low- and middle-income countries, where CC is the third most common cancer among women. According to GLOBOCAN 2020, CC is the second most common cancer in Mexico with 9,439 new cases and 4,335 deaths per year. The Federation of Gynecology and Obstetrics (FIGO) staging system classifies CC in four stages, I-IV, which in turn are subdivided in various subtypes; this classification is mainly based on surgery, pathologic analysis and imaging (2).

The expression of certain circulating biomolecules is modified when a disease is established, having a great potential to detect and predict the disease as well as to identify the response to different treatments (3-5). For CC, there are several studies showing biomolecules, proteins, non-coding RNAs (ncRNAs), and circulating DNA (cDNA) with a great potential to be useful biomarkers for diagnosis, prognosis, and to determine the response to treatments currently used in medical attention for CC. Although certain of these protein biomarkers may also function as biomarkers for other HPV-related cancers, previous studies showed that the combined use of these molecules increases their potential as biomarkers for this disease (6-8). Although these biomarkers have a sensitivity very similar to that of colposcopy and p16 (Table I) (9-29), a significant advantage is that it is a minimally invasive methodology, having a very important impact on the number of women who would be screened with this test. The aim of the present study was to review the literature related

to these potential biomarkers, emphasizing improved results as biomarkers when these molecules have been analyzed in combination.

The information was searched in Pubmed and in academic Google. The criteria followed to search the literature were the following: Circulating biomarkers, CC, ncRNAs and cDNA. In addition, biomarkers for HPV-associated cancers and public spending in Mexico for the treatment of CC were searched. The inclusion criterion was full access to the reviewed articles. Those articles that could not be accessed were excluded from the review.

2. Proteins

The search of circulating proteins as potential biomarkers in pathologies such as cancer has been addressed for several decades. The objective is clear, to diagnose and monitor the diverse types of cancer in a minimally invasive way; however, this search has not been easy and even when there are proteins currently used in the medical attention, their sensitivity (the ability to detect a disease in patients in whom the disease is actually present) and specificity (the ability to rule out the disease in patients in whom the disease is actually absent) is relatively low (9). To date, the most common circulating cancer marker proteins used in medical attention for distinct types of cancer are: i) The squamous cell carcinoma antigen (SCC-A), ii) The carcinoembryonic antigen (CEA), iii) The α-fetoprotein, iv) The β-subunit of human chorionic gonadotropin, v) Lactate dehydrogenase and vi) The cancer antigen 125 (10). Regarding CC, massive analyses have revealed groups of circulating proteins differentially expressed in this disease with a great potential to be used as biomarkers. Notably, the use of two or more of these proteins has been revealed to considerably increase their sensitivity and specificity (Table II) (30,31).

In the 1970s, the SCC-A was identified by using the hybridoma technique in SCC of human uterine cervix (11). SCC-A is a serpin that comprises two nearly identical proteins (45 kDa), SCC-1 and SCC-2, which possess unique proteinase inhibitory properties (10,11). SCC-1 exerts an anti-apoptotic action through the inhibition of chymotrypsin and cathepsin L. The mechanism of protection of tumor cells from apoptosis involves the inhibition of the caspase-3 activity and/or upstream proteases. SCC-2 inhibits cathepsin G and mast cell chymase, thus protecting epithelial cells from these proteases-induced inflammation (32).

Increased serum SCC-A levels were observed in more advanced SCC stages (in 28-88% of the patients) allowing the use of SCC-A as diagnostic and prognostic biomarker for this cancer subtype (30-32). Differences in the percentage of SCC-A detection were attributed to various factors, such as the histological grade and the cutoff in the SCC-A serum concentration. Although numerous years have passed since its discovery, the clinical use of SCC-A remains under debate, for the increase on its expression has been reported in patients with SCC of the esophagus, lung, head, neck, and in anal canal and uterine cervix, as well as in patients with several non-malignant skin lesions, such as pemphigus and renal failure. Regarding this, the exposure to TNF-α significantly increased the production of SCC-A in normal human epidermal keratinocytes (33).

In addition to SCC-A, other potential circulating biomarkers have been identified. Mitsuhashi *et al* (21) described that the serum YKL-40 level was elevated in both SCC and adenocarcinoma. YLK-40 is a glycoprotein member of the glycosyl hydrolase 18 family; it is secreted by active macrophages, chondrocytes, neutrophils and synovial cells. Recent studies suggested that YLK-40 plays a role in the inflammation process and tissue remodeling (34-36). This molecule appears to be a favorable CC biomarker in both SSC and adenocarcinoma subtypes, and it appears to be more specific than SCC-A and CA125. Previous findings demonstrated that serum YKL-40 level is increased in several solid tumors with a variety of histological types (37,38). This protein is a biomarker associated with inflammation and, despite this, it could be a correlation between the C-reactive protein (CRP) and YKL-40 (39). YKL-40 serum appears to be more a non-specific biomarker of inflammation, since its expression was higher than that of CRP, allowing to discriminate patients with CC from tumor-free individuals. In addition, YKL-40 appears to be an improved serum biomarker for adenocarcinomas detection than CA-125 exhibiting 78 and 68% sensitivity for all grades and for stage I tumors, respectively (21). Although it does not appear to be an ideal biomarker due to its relative low sensitivity to detect CC, the receiver operating characteristic (ROC) and area under a ROC curve (AUC) analysis revealed that YKL-40 discriminates healthy individuals from patients with CC. Similarly, the YKL-40 levels were identified to be a poor prognostic variable for relapse of the disease (40).

Sheng *et al* (41) examined the clinical value of serum high mobility group box chromosomal protein 1 (HMGB1) levels in the early diagnosis of recurrent cervical SCC and compared them with the values obtained for SCC-A, cytokeratin fragment 21-1 (CYFRA) and CEA. HMGSB1 is a nuclear DNA-binding protein able to regulate transcription and is involved in organization of DNA, playing a role in several cellular processes including inflammation, cell differentiation and tumor cell migration. In the present study, serum HMGB1 levels-in patients with recurrent CSCC-were significantly higher than in patients with non-recurrent disease and healthy controls. The combination of the HMGB1 expression with other biomarkers such as SCCA, CYFRA21-1 and CEA increased the sensitivity of HMGB1 to detect CC and serial combinations of these markers also increased the specificity. Relatively high serum expression levels of HMGB1 were inversely correlated with disease-free survival and overall survival.

A proteomic screening carried out by Chen *et al* (42) in 10 healthy control women and 39 patients with CC, before and after surgery, identified three peptide biomarkers, distinguishing patients with CC from individuals without cancer as well as preoperative patients with CC from those who had already been subjected to surgery. TKT and FGA peptides were upregulated in CC and preoperative patients, whereas the APOA1 peptide region was downregulated. Meanwhile, collagen triple helix repeat containing 1 (CTHRC1), a protein that may play a role in the cellular response to arterial injury through the involvement in vascular remodeling, was evaluated as another potential serum marker for CC detection, particularly for SCC. Xu *et al* (22) studied the CTHRC1 expression in three different groups [individuals without cancer, SCC, and cervical intraepithelial lesions (CIN)], demonstrating its

Table I. Comparison of sensitivity and specificity among biomarkers and other CC detection methods.

Method	Sensitivity	Specificity	Technique strengths	Limitations	(Refs.)
Papanicolau	51% (37-84%) 17% 13% 61% 72%	98% (86-100%) 83%	-Success in developed countries. -Relative high specificity. -Well-characterized screening method.	-Unsuccessful test in developing countries. -Relative low sensitivity. -A high rate of false negatives. -The effectiveness depends on the technician performing it. -A high rate of false negatives (56%) -A high rate of false negatives (72%) -False negatives: 25% -Expensive for Health systems. -High invasive methodology.	(9-13,17)
Colposcopy	87% 83-98%	48-66%	-Relative high sensitivity. -It allows to observe the presence of a suspicious area of injury, as well as to limit its extension and its severity to take the biopsy. -Few false negatives	-Relative low specificity, having relatively high false positives. -Colposcopist experience is need. -Expensive for Health systems. -High insasive methodology.	(10, 15-17)
Liquid cervical cytology	75%	86%	In the United Kingdom the NHS cervical screening program has been estimated to prevent ~80% of deaths from cervical cancer	Although, liquid based systems are routinely used in many countries, the cost of the technique limits its utility in developing countries	(20)
P16 detection	88%		Improves HPV DNA testing and cervical cytology diagnosis.	-A biopsy is needed. -High invasive methodology.	(19)
PCR	45% 60-80%		-PCR allows to detect the virus type and the hybrid capture test generically detects high and low risk types. Relative high sensitivity.	-The PCR test does not provide information regarding the type of lesion and has higher cost than conventional colposcopy. -A biopsy is needed.	(11,14,18)
Protein biomarkers	• YLK-40: 78% • CTHRC1 and SCC-Ag: 87%	40% 84% 92%	-The combined expression of these potential biomarkers results in a relatively high sensitivity and specificity.	-The sensitivity and specificity of these biomarkers may change between ethnicities.	(21-24)
microRNAs (combined)	•PIGF and Flt-1: 70% •VEGF: 87%	76% 70 and 95%	-Early LGCL and HGCL, and CC detection. -Prognosis determination.		(25,26)
LncRNAs (combined)	73 and 96%		-Prediction of response to treatments.		(27,28)
E7 HPV16/18 cfDNA	AUC=88% AUC=94% 100%	88%	-Minimally invasive methodology.		(29)

Table II. Circulating proteins as potential biomarkers for cervical cancer.

Protein biomarker	Diagnostic biomarker	Prognostic biomarker	Type of cervical cancer	(Refs.)
SCC-A	X		SCC	(30-32)
CEA	X		SCC and adenocarcinoma	(30,31)
CA-125	X		Adenocarcinoma	(30,31)
YKL-40	X		SCC and adenocarcinoma	(21)
HMGB1		X	SCC	(33)
TKT	X		SCC	(34)
FGA	X			
APOA1	X			
CTHRC1	X		SCC	(22)
M-CSF	X	SCC and adenocarcinoma		(35)
VEGF	X			
ACTN4		X	SCC	(36)
PIGF	X		SCC	(23)
VEGFR1	X			

X: The corresponding biomarker is useful for the stated purpose. SCC, squamous cell carcinoma; CEA, carcinoembryogenic antigen; CA-125; HMGB1, high mobility group box chromosomal protein 1; CTHRC1, collagen triple helix repeat containing 1; M-CSF, macrophage-colony stimulating factor; VEGF, vascular endothelial growth factor; PIGF, placental growth factor.

overexpression in SCC relative to CIN and individuals without cancer. The ROC curve showed an AUC value for CTHRC1 and SCC-Ag of 0.665±0.034, and 0.878±0.027 respectively; the sensitivity and specificity for these biomarkers were 57 and 85% (CTHRC1), and 78 and 86% (SCC-Ag), respectively. Importantly, the combined analysis of CTHRC1 and SCC-Ag considerably increased the AUC value (0.879±0.027) and the sensitivity (87%) and specificity (84%). The aforementioned study strongly suggested the potential use of CTHRC1 as a novel prognostic and metastatic biomarker for SCC, whose potential as a biomarker increased considerably when combined with SCC.

It is well documented that activation of Macrophage-Colony Stimulating Factor (M-CSF) and vascular endothelial growth factor (VEGF) is involved in the pathogenesis and spread of distinct types of cancer, including CC. Regarding this, Sidorkiewicz *et al* (24) examined the M-CSF and VEGF plasma levels and compared them with those of CA-125 and SCC-A in three groups of patients: i) The CC group (patients with either SCC or adenocarcinoma), ii) The cervical dysplasia group and iii) The control group. The median levels of M-CSF and VEGF as well as those of CA-125 and SCC-A were significantly different in the three groups relative to the control group. The sensitivity and specificity for VEGF and SCC-Ag were of 82 and 76%, and 81 and 74%, respectively. In the adenocarcinoma group, the VEGF sensitivity and specificity were respectively of 87 and 76% (24). The results indicated a possible clinical applicability for these proteins and a relatively high diagnostic power for the M-CSF, VEGF, CA-125 and SCC-Ag combination. Similarly, the combined analysis of α-Actinin 4 (ACTN4) and SSC-A is a promising serological examination for CC detection. ACTN4 plays an essential role in regulating cellular signaling pathways correlated with various types of cancer progression and poor patient prognosis, involved in the invasion and metastasis of colorectal, pancreatic and ovarian cancer. Its principal function is by regulating cell invasion due to its participation in the epithelial-to-mesenchymal transition; however, it is also involved in controlling the cancer stem cell properties and chemoresistance in CC. Zhu *et al* (43) demonstrated the circulating and tumor ACTN4 overexpression in patients with CIN3 or more advanced stages. In addition, the ACTN4 mRNA was also overexpressed in CC tissues and in tissues with advanced FIGO stages, larger tumor sizes, and positive lymph node metastasis. In conclusion, ACTN4, in combination with SCC-Ag, is a potential biomarker for the diagnosis and prognosis of patients with CIN3 or more advanced stages.

Yang *et al* (23) proposed that circulating placental growth factor (PIGF) and its receptor VEGFR-1 (Flt-1) can serve as possible valuable diagnostic biomarkers for CC, and their combined use increased the potential to diagnose patients with early CC. A total of 44 preoperative patients with CC, 18 cases with CIN, and 20 controls were studied and both PlGF and Flt-1 were significantly increased in the CC group when compared with that with CIN or without cancer. PlGF presented a relatively high power to detect CC with a 61% sensitivity and an 89% specificity; meanwhile, Flt-1 showed a 50% sensitivity and a 92.11% specificity. Remarkably, the combined use of PlGF and Flt-1 increased the CC diagnosis (sensitivity of 70% and specificity of 92%) (23).

Summarizing, different circulating proteins are differentially expressed in patients with CC relative to individuals without cancer, having a great potential to be used in clinical diagnosis and even more when two or more proteins are analyzed in a combined manner. Importantly, not only circulating proteins have been identified as biomarkers for CC but also microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), as well as cDNA.

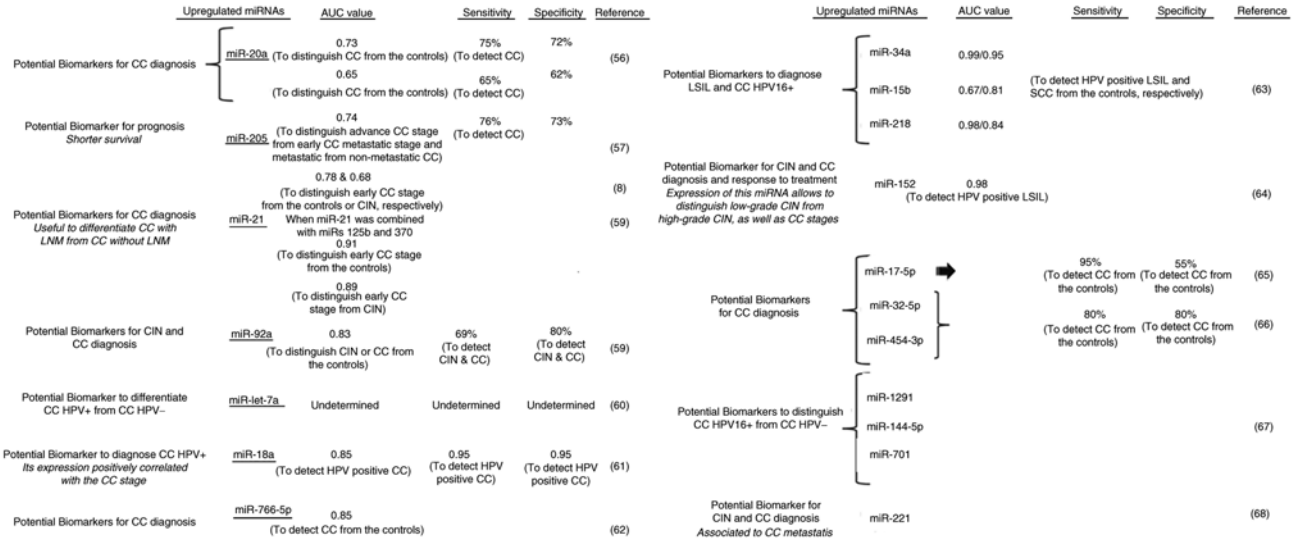

Figure 1. Upregulated miRNAs. These group of miRNAs have the potential to be biomarkers to distinguish CIN, LGCLs or CC from the controls. MiRs 18 and 17-5p showed the highest sensitivity for CC diagnosis (~95%). LGCLs, low-grade cervical lesions; CIN, cervical intraepithelial lesions; CC, cervical cancer; miRNA or miR, microRNA.

3. ncRNAs

ncRNAs are RNA molecules, which are not translated into a protein, including transfer RNAs, ribosomal RNAs, small non-coding RNAs (snRNAs) and lncRNAs (44). snRNAs and lncRNAs regulate numerous biological functions and their expression is finely regulated at different stages of development of organisms to fulfill very particular functions (45,46). Numerous lines of evidence indicate their involvement in cancer, specifically in CC, and their differential expression, mainly in blood, has been related to diagnosis, prognosis, and treatment response of patients with CC, as described below.

MiRNAs. MiRNAs are snRNAs of 18-34 and 80-100 nucleotides (nt) (47-49) in size, which control gene expression by inhibiting protein translation, by regulating gene transcription and the expression and/or the function of lncRNAs (50-52). Notably, circulating miRNAs act as Toll-receptors ligands to activate intracellular signaling pathways resulting in the control of cellular response in other organs and tissues (53).

To date, numerous circulating miRNAs have been identified as possible biomarkers for low-grade squamous intraepithelial lesions (LSIL), CIN and CC and most of them demonstrate higher AUC values and a higher sensitivity and specificity than proteins. Notably, both sensitivity and specificity to detect CC by miRNAs is relatively high and they were considerably increased when analyzed in combination (6-8,54-72) (Figs. 1-3). The majority of miRNAs have the potential to be biomarkers for CIN and CC diagnosis and only certain for prognosis (Fig. 3). MiRs 34a and 218 are particularly important, since they allow to distinguish LSIL and CC HPV16$^+$ from healthy women (60) (Fig. 1). Their clinical use in LSIL detection-in a minimally invasive form-would have a huge impact on public health in countries where CC remains a public health problem, such as Mexico.

A very interesting study showed the miR-221-3p enrichment in exosomes, which were secreted by CSCC and captured by human lymphatic endothelial cells (HLECs), resulted in their migration promotion, tube formation, lymphanogenesis induction and LN metastasis in CSCC patients. These processes appear to be regulated, at least partly, by targeting vasohibin-1, leading to the ERK/AKT pathway activation in HLECs (65).

To date, there are several circulating miRNAs that can be used for the LSIL, CIN and CC diagnosis and certain of them are specific to differentiate HPV$^+$ CC from the negative one. Importantly, certain miRNAs render it possible to identify CC metastasis to the lymph nodes.

LncRNAs. LncRNAs are ≥200 nt RNAs with very complex secondary structures and a myriad of cellular functions: i) Maintaining the chromatin structure and regulating gene transcription (73), ii) They are molecular scaffolds for several factors involved in transcription control (74) and iii) They are miRNAs sponges (75). These type of RNAs have been detected in body fluids and have been associated with cancer, including CC; however, the information regarding circulating lncRNAs participating in CC is very scarce. Sun *et al* (27) found that lncRNAs HOTAIR, PVT1, XLOC_000303 and AL592284 are overexpressed in the blood serum of patients with CC when compared with the controls. The analysis of these four lncRNAs together improved the AUC value: 0.875. In a similar way, the analysis of the overexpressed lncRNAs CCAT2, LINC01133 and LINC00511 by including the SCC-A, increased the AUC value to 0.94 (28). Meanwhile, the expression of HOTAIR was increased in patients with CC relative to the controls and this correlated with numerous clinical aspects as well as with tumor recurrence and short overall survival (76). The identification of more circulating lncRNAs as potential CC biomarkers will definitely have a noticeable impact on the diagnosis, prognosis and response to treatments of this type of cancer. In addition, the discovery of the

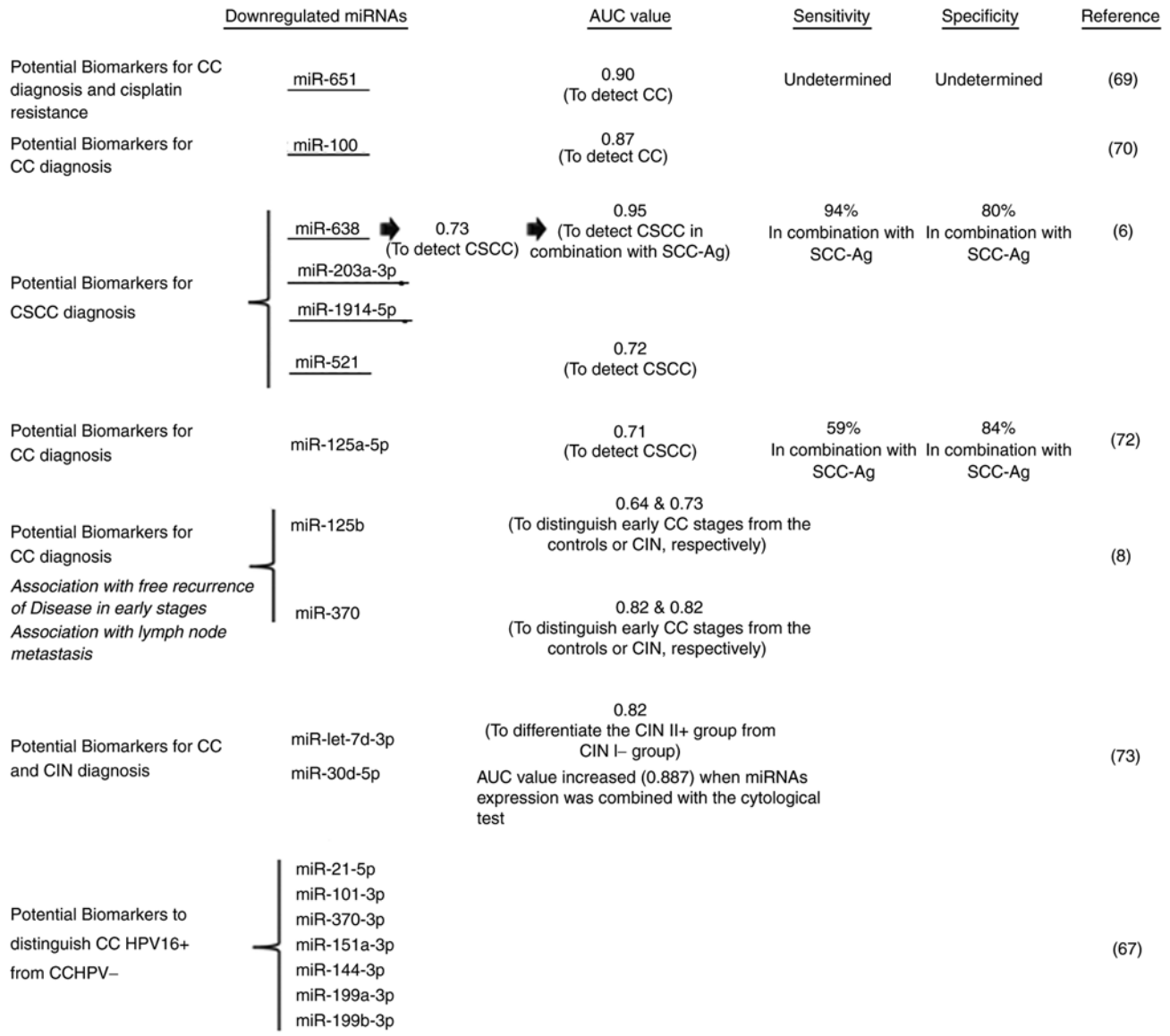

Figure 2. Downregulated miRNAs. miR-651 showed the highest AUC value to detect CC and it was also positively correlated with cisplatin resistance. Meanwhile, miR-638 had a very high sensitivity for CSSC diagnosis, but only when its expression was analyzed with that of SCC. miRNA or miR, microRNA; AUC, area under the curve; CC, cervical cancer; CSCC, cervical squamous cell carcinoma.

functions performed by these RNAs in time shall allow the identification of therapeutic targets in the future.

4. The estrogen effect on the expression of biomarkers

There is a close relationship between gynecological cancers and alterations in hormone-mediated regulatory pathways, modifying gene expression. Most information is related to protein biomarkers and some of them were regulated by estrogens in tissues: SCC-Ag, C-125, VEGFA and ACTN4, and only circulating HMGB1 and YKL-40 were regulated by estrogens (77-81). Notably, the circulating miR-21 expression was downregulated by estrogens (82).

To the best of our knowledge, for the remaining biomarkers there is no evidence indicating changes on their circulating expression in response to estrogens; however, this does not mean that their expression cannot be regulated by this hormone, since most of them show a close relationship with the expression of estrogen receptors.

5. cDNA

cDNA was discovered several years ago (82) and its relationship with cancer was later identified (83). The total cDNA concentration is considerably lower in healthy individuals than in patients with cancer (84) and it varies depending on the cancer type (85). Notably, diverse subtypes of cfDNA can be found in circulation, such as mitochondrial DNA and extrachromosomal and single-stranded DNA, viral, bacterial, and food-derived (86). The majority of free cDNA is originated from the nucleus and it is packaged in mono- or oligonucleosomes, but most free cDNA is associated with exosomes.

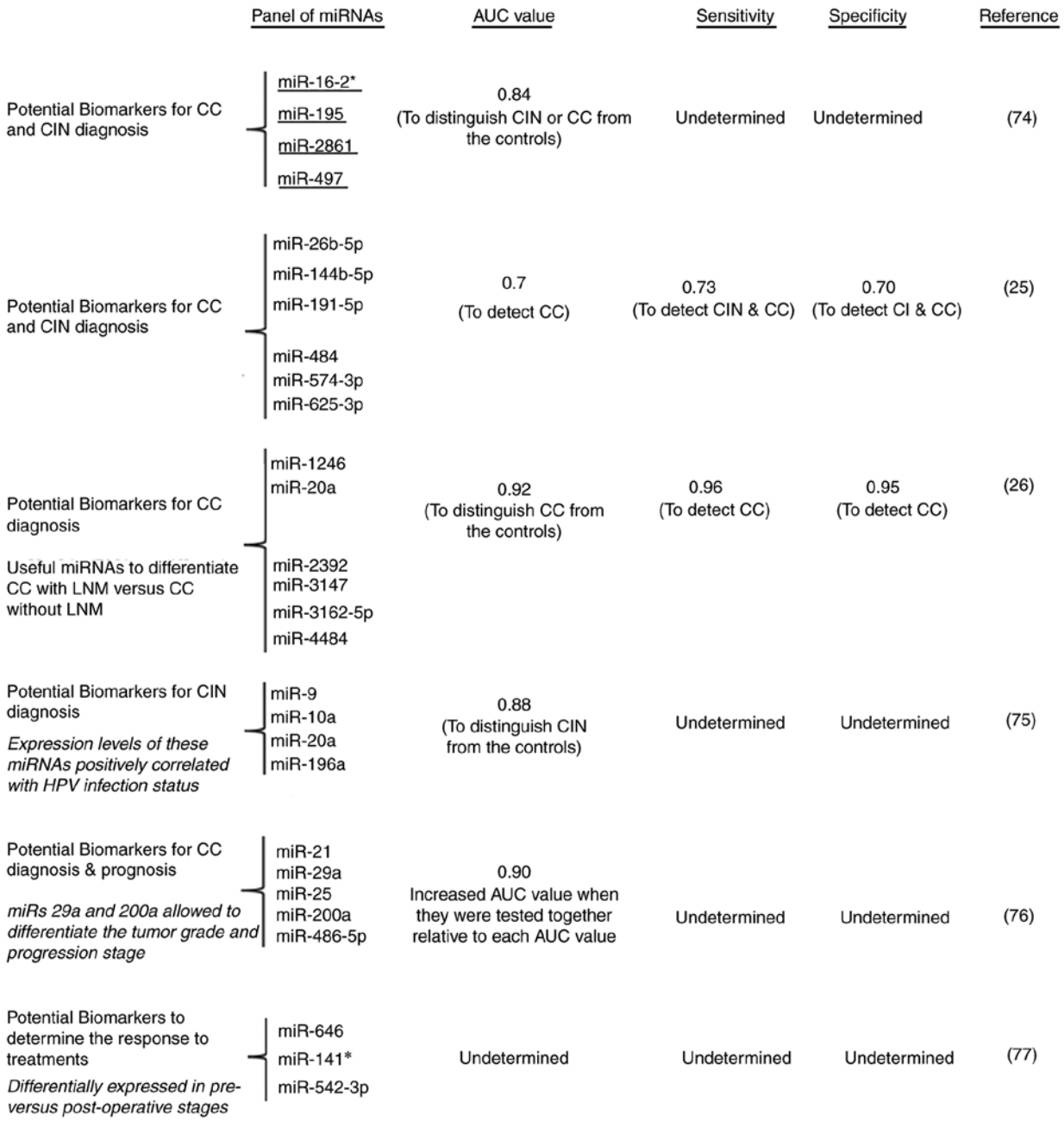

Figure 3. Combined miRNAs analysis. The combined analysis of the miRNAs expression showed their potential use to diagnose CIN and CC, as well as to CC prognosis and to determine the response to treatments of patients. Importantly, the combination of two or more miRNAs considerably increases the sensitivity and specificity to detect and/or predict the response of patients to treatments. miRNA or miR, microRNA; CIN, cervical intraepithelial lesions; CC, cervical cancer; AUC, area under the curve.

Free cDNA release does not correlate with the necrosis and apoptosis levels, but it does correlate with the percentage of cells in G1 phase (87). Perhaps most importantly, cDNA appears to have various cancer-related functions (88-90).

Previous studies showed that HPV-cDNA detection positively correlates with low-grade cervical lesions (LGCL) and high-grade cervical lesions (HGCL), CIN and CC, tumor grade, and with the genomic HPV insertion, which is associated with a poor patient's prognosis (91-98) (Table III). Importantly, Rungkamoltip *et al* (29) showed a 100% sensitivity and 88% specificity to detect E7 HPV16/18 cfDNA by using the amplification-by recombinase polymerase-in combination with lateral Flow strip. In addition to cDNA, the presence of specific cDNA mutations allows to detect CC from healthy individuals and positively correlates with disease progression and with a shorter progression-free survival, as well as with the overall survival of patients with metastatic relapse CC compared with the controls (patients without any detectable of these mutations) (99).

Very importantly, cDNA detection allows to differentiate among LGCL and HGCL, and CC from patients with non-HPV dependent CC as well as from individuals without CC. LGCL detection by means of cDNA is very promising since the early detection of this type of lesions can significantly prevent their progression to cancer. In addition, cDNA concentration appears to be useful for monitoring treatment response and patient's prognosis.

Table III. cDNA expression levels.

HPV-cDNA Detection (%)	Overexpression in CC patients vs. the controls or early stages of the disease	Related to tumoral grade and/or progression	Associated with treatment response	Associated with poor prognosis	(Refs.)
12% of the HPV-positive patients with CC		• CC patients without detectable cDNA did not progress		• 50% of the patients were in stage IIB or in recurrent metastasis	(77)
Undetected in the controls or HPV-negative patients	√			• Three-fold higher expression in patients with metastases • Detection of cDNA predisposed to develop metastases	
Undetermined	√		• Decreased of the cDNA expression levels; 83% of patients responded to treatment		(27)
Undetermined	√	• cDNA expression higher in stage II-III patients than in stage I or CIN patients or controls			(78)
HPV 16: 50% of the CC patients HPV 18: 6% of the CC patients and 1% of the controls	√		• Decreased of the cDNA expression levels; 76% of patients responded to treatment		(79)
51% of patients with squamous intraepithelial lesions. More than half of these patients were positive for at least one high-risk HPV	√				(80)
85% of patients with CC	√	• The cDNA expression levels increased according to the degree of malignancy			(81)
Undetermined	Undetermined		• After the treatment, the cDNA levels were undetectable in 68% of patients • Only one patient showed recurrence. • 46% of the patients showed no residual disease (at three months follow-up)	• After treatment, relative high cDNA expression levels were associated with metastases development (in 50% of the patients)	(82)

Table III. Continued.

HPV-cDNA Detection (%)	Overexpression in CC patients vs. the controls or early stages of the disease	Related to tumoral grade and/or progression	Associated with treatment response	Associated with poor prognosis	(Refs.)
Undetermined	Undetermined			• The HPV-ctDNA detection correlated with a lower disease free survival and overall survival	(82)
E7 HPV16/18 cell free DNA highly specific to detect CC (100% sensitivity and 88% specificity)	√				(83)

CC, cervical cancer; SCC, squamous cell carcinoma; cDNA, circulating DNA.

Table IV. Kyoto Encyclopedia of Genes and Genomes analysis.

Identifier	Signaling pathway	Number of genes involved	Cellular processes potentially altered
Ko04151	PI3K-Akt signaling pathway	3 (FLT1; VEGFA; CSF1)	Cell proliferation. Angiogenesis. DNA repair.
Ko04066	HIF-1 signaling pathway	3 (LDH; FLT1; VEGFA)	Angiogenesis. Pyruvate to Lactate.
Ko04015	Rap1 signaling pathway	3 (FLT1; VEGFA; CSF1)	Defective angiogenesis. Smg cross-talk. Cell adhesion, migration, polarity. Proliferation and survival. Gene activation.
Ko04010	MAPK signaling pathway	3 (FLT1; VEGFA; CSF1)	Proliferation. Differentiation.
Ko04014	Ras signaling pathway	3 (FLT1; VEGFA; CSF1)	Apoptosis. Cell-cycle arrest. Cytoskeleton organization. Cell motility. Cell survival, growth, migration. Cell cycle progression. Transcription. Endocytosis. Gene expression. Cell-cell junctions. Cytoskeletal remodeling. Cell spreading and migration.
Ko04510	Focal adhesion	3 (FLT1; VEGFA; ACTN1)	Actin polymerization. Cell survival.
Ko05323	Rheumatoid arthritis	3 (FLT1; VEGFA; CSF1)	Angiogenesis.

VEGF, vascular endothelial growth factor; ACTN4, Actinin 4; CSF, colony stimulating factor.

6. Putative molecular mechanisms altered by the CC biomarkers

Making a prediction of which mechanisms will be regulated by specific circulating molecules-only based on their canonical functions-is a high-risk task. This is because the few studies that have focused on studying the action of circulating molecules have elucidated mechanisms of action somewhat unexpected and that have nothing to do with the previously reported effects.

In general, it is known that exogenous miRNAs regulate gene expression through the canonical pathway, involving the binding to their target mRNA; however, Fabbri *et al* (100) revealed that miRs-21 and -29a function by a different mechanism. The aforementioned study demonstrated the interaction of these miRNAs with Toll-like receptor (TLR) 7 and TRL8 in cells from the immune system. Notably, the binding of miRs-21 and -29a to these receptors triggered a prometastatic inflammatory response, resulting in the tumor growth induction and metastasis. Thus, this is the first indication that

Table V. DIANA miRPath v.3.

Kyoto Encyclopedia of Genes and Genomes pathway	P-value	Genes	MicroRNAs	Cellular processes potentially altered
Proteoglycans in cancer	$1.06x10^{-24}$	165	26	Angiogenesis. Proliferation and survival. Tumor cell migration and invasion.
Pathways in cancer	$7.31x10^{-15}$	299	28	Carcinogenesis. Genomic damage. Resistance to chemotherapy. Insensitive to anti-growth signals. Failed repair of genes. Sustained angiogenesis. Evading apoptosis. Proliferation. Block of differentiation. Tissue and metastasis.
Renal cell carcinoma	$8.81x10^{-13}$	61	25	Increased nutrients and oxygen. Glucose transport. Angiogenesis. Autocrine growth stimulation. Cell proliferation, migration, invasion. Genetic alterations. Ubiquitin mediated pathway.
Prostate cancer	$3.00x10^{-11}$	81	27	Anti-apoptotic genes. Proliferative genes. G1/S progression. Cytoskeleton remodeling. Inhibition of apoptosis. Loss of growth inhibitory effects of TGFβ. Failed repair of genes.
Colorectal cancer	$3.43x10^{-11}$	58	26	Genetic alterations. DNA repair genes. Anti-apoptosis. Proliferation. Loss of growth inhibitory effects of TGFβ. Karyotypic instability. Impaired G1 cycle arrest. Reduced apoptosis.
Viral carcinogenesis	$3.02x10^{-12}$	156	26	Proliferation. Viral infectivity and replication. Proliferation. Inhibition of apoptosis. Survival. Inhibition of p53-mediated apoptosis. Inhibition of nucleotide excision repair. Induction of FasL. Mitochondrial dysfunction. Alteration of host cellular gene expression. Stimulation/inhibition of cell proliferation. Inhibition of mitogenic signaling. Growth retardation. Inhibition of transcriptional activation by p53. Basal transcription factors. Transformation. Suppression of immunoresponse. Decreasing differentiation.
Fatty acid metabolism	$2.12x10^{-9}$	37	33	Citrate cycle.
Transcriptional misregulation in cancer (hsa05202)	$5.14x10^{-9}$	147	45	Differentiation resistance. Self-renewal of T cells. Proliferation, cell survival. Myeloma adhesion to bone marrow stroma. Cell cycle progression. Inhibition of apoptosis. Repression of tumor suppressors. Escape from growth inhibition, senescence, apoptosis. Low radiosensitivity of tumor cells. Interactions with ECM, migration, invasion. Karyotypic instability, impaired G1 cycle arrest, reduced apoptosis.
Endocytosis	$6.80x10^{-9}$	172	43	Lysosome. TGF-beta signaling pathway. Phosphatidylinositol signaling System.
Hippo signaling pathway	$6.80x10^{-9}$	125	45	Pro-apoptotic genes. Anti-apoptotic genes.Pro-proliferation genes. Cell contact inhibition Organ. size control. Adherens junction. Tight junction.

Table V. Continued.

Kyoto Encyclopedia of Genes and Genomes pathway	P-value	Genes	MicroRNAs	Cellular processes potentially altered
Non-small cell lung cancer	$8.62x10^{-9}$	53	39	Tumour progression. Impaired G1and G2 arrest. Reduced apoptosis. Genomic instability. Metastatic squamous cell carcinoma. Metastatic adenocarcinoma.
Prostate cancer	$8.62x10^{-9}$	82	45	Cell cycle. Apoptosis inhibition. Tumor growth. Androgen and estrogen metabolism.
Acute myeloid leukemia	$1.05x10^{-8}$	55	39	Anti-apoptotic genes. Proliferative genes. AML1 target genes. Block of differentiation.
Cell cycle	$2.15x10^{-8}$	105	44	Ubiquitin-mediated proteolysis. Apoptosis. DNA damage checkpoint. DNA biosynthesis.

Table VI. Panel of biomarkers.

Low-grade squamous intraepithelial lesions	miR-34a, miR-15b, miR-18 and circulating DNA
Early CC stages from the controls	miR-21, miR-125b and miR370
CC detection	Proteins: Collagen Triple Helix Repeat containing 1 and squamous cell carcinoma antigen Long non-coding RNAs: CCAT2, LINC01133 and LINC00511
Cisplatin resistance	miR-651

miR, microRNA; CC, cervical cancer.

miRNAs function as paracrine agonists of TLRs to regulate tumor environment.

Kyoto encyclopedia of genes and genomes (KEGG) analysis. Based on canonical functions reported for the proteins proposed as biomarkers for CC, KEGG analysis showed their involvement mainly in the control of signaling pathways, such as PI3K-Akt, HIF-1, and Rap1, among others (Table IV). The cellular processes that may be modified by changes in these signaling pathways were cell proliferation, adhesion, migration, cytoskeleton remodeling and gene expression regulation (Table IV).

DIANA miRPath analysis (Tarbase). Changes on expression of miRNAs and/or on their function could alter numerous signaling pathways and cellular processes, which is related to their ability to regulate several mRNAs in the same cell. Secretion of miRNAs to blood serum and target organ recognition could mainly alter proteoglycans in cancer, pathways in cancer, renal cell carcinoma, viral carcinogenesis, among others; these signaling pathways control cellular processes related with hallmarks of cancer (Table V).

Numerous functional studies are necessary to know the mechanism(s) of action of each of these molecules separately and/or together, and what 'benefit' the tumor obtains by releasing these molecules.

7. Expression of circulating biomarkers in other HPV-related cancers

The aforementioned biomarkers are postulated as a favorable tool for both the detection of cervical lesions and cancer; however, various studies have shown the expression of some of these biomarkers in other types of HPV-related cancers. Their expression has been detected mainly at the tumor level and to a markedly lesser extent as circulating molecules. SCC-A is the most studied biomarker and is overexpressed in distinct squamous cell cancers, including the following: Esophagus, lung, head, neck, and in anal canal, and uterine cervix, as well as in vulvar and penile carcinoma (101-105). Meanwhile, YKL-40 has demonstrated high tissue levels in anal carcinoma and it has been detected in the plasma of patients with esophageal cancer (106) and CA-125 showed relatively high circulating levels in vulvar carcinoma (107). In addition, CA-125 had an increased expression in oropharyngeal cancer (108) and relatively high VEGFA expression levels were detected in vulvar carcinoma (109) and oropharyngeal cancer (110). Although PIGF was detected in oropharyngeal cancer, its expression was not related with the malignancy of this cancer type (111).

In the case of ncRNAs, to the best of our knowledge only miR-205 and HOTAIR have been related with head and neck (112), and oropharyngeal cancers (113), as well as with cervicovaginal lavage specimens, respectively (114).

8. How feasible is it to use a test of this type in the heath sector of undeveloped countries?

Even though the Mexican Institute of Social Security has a substantial cost for the detection, follow-up and treatment of CC, this cancer type remains the second most common in Mexican women after breast cancer. A previous study carried out by Granados-García *et al* (17) revealed the high cost of evaluating a patient's condition regarding CC by cytology, colposcopy, biopsies, and pathology, as well as by diagnostic tests and treatments for cervical intraepithelial neoplasia grade II and III (CIN 2/3) and CC. The aforementioned study identified that the cost to perform 2.7 million cytology tests was nearly 38 million dollars, representing 26.1% of the total program cost (145.4 million). False negatives account for nearly 43% of the program costs. According to the aforementioned results, it was concluded that the low sensitivity of the cytology test generates high rates of false negatives, resulting in high institutional costs from the treatment of undetected CC cases.

In accordance with the aforementioned studies, the establishment of a panel of biomarkers with high sensitivity and specificity would be a great molecular tool to improve the diagnosis and treatment of women with LGCLs, HGCLs and CC. An increase in the detection of women with LGCLs and HGCLs-by this panel-may decrease the number of women progressing towards CC.

9. The best biomarkers panel, according to the authors' point of view

From the authors' point of view, the biomarkers that would have a greater impact on women and health sector are those detecting both LGCLs and HGCLs. Early detection of these type of lesions would allow early treatment of women and this would considerably decrease the progression towards cancer. At this point it is important to mention that the use of this biomarker panel will increase the power of detection, prognosis and response to treatments (Table VI).

10. Conclusions and future directions

As observed, early CC detection is a crucial factor to effectively treat low-grade CC lesions and thus avoid the transition to cancer; therefore, the establishment of molecular tools that allow performing this task is imperative. Currently, there are diverse biomolecules-particularly ncRNAs-which have a high sensitivity and specificity to detect LGCLs as well as CC, thus the establishment of these biomarkers for their use in the clinical studies to detect LGCLs and CC is crucial. In addition, the biomolecules that enable us to know the response to treatments is also very important and, in the same way, it should be part of the molecular tools used in the medical attention.

Collectively, the biomarkers found to date have great potential to be used as clinically useful biomarkers for detection and response to treatments. Further studies are needed to establish which are the ones that will best support the medical attention.

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Authors' contributions

RREG searched and organized the information and wrote the manuscript. MGS reviewed the last version of the manuscript. MAVF reviewed and corrected the information of the last version of the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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