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***Development and validation of a non-invasive screening and diagnosis test for endometrial cancer through the new Next Generation Sequencing techniques of the circulating tumor DNA (Liquid Biopsy).***


**Relatore**

**Prof. Attilio Di Spiezio Sardo**

**Candidato**

**Dott.ssa Giuseppina Esposito**

**matr.** **DR993746**


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Simple Summary

There is currently no blood-based marker in routine use for endometrial cancer patients. Such a marker could potentially be used for early detection, but it could also help to track tumor recurrence following hysterectomy. This study was designed to determine if tumor-associated mutations could be detected in cell-free DNA from the peripheral blood of endometrial carcinoma or atypical hyperplasia patients. Next-generation sequencing was performed on cell-free DNA extracted from plasma obtained from a peripheral blood draw at the time of hysterectomy/hysteroscopy and the matching tumor DNA from 85 patients with endometrial carcinomas or atypical hyperplasia. At least one pathogenic mutation in plasma samples was detected in 70/77 (94%) of the tumors analyzed, the detected mutation on plasma was the same of the one detected in solid tumor in 50/77 (65%) of cases. The cfDNA expression in plasma do not relate with grading (p>0,05) but is associated with myometrial infiltration (chi-square 25; p=0,001). The presence of cfDNA mutation of PTEN (chi-square 25; p<0,001) and PIK3RI (chi square 22; p<0,001) in plasma related with grading G3 and >50% infiltration. The presence of cfDNA mutation of CTCF (chi-square 12; p<0,01) and BRAF (chi square 18; p<0,001) related with >50% infiltration. ZFHX3, P53, PTEN was associated with diagnosis of endometrioid cancer while KMT2C was associated with atypical hyperplasia. These results demonstrate that mutations in genes relevant to endometrial cancer can be identified in the peripheral blood of patients at the time of surgery or of hysteroscopy for patient who undergo conservative management. Future studies can help to determine the post-operative time course of mutation clearance from the peripheral blood and if mutation re-emergence is predictive of recurrence.

## ***Introduction***

Endometrial cancer (EC) is the fourth leading cancer in women from developed countries. In Europe, the number of new cases was about

100,000 in 2012, with an incidence of 13.6 per 100,000 women [1]. This tumor originates in the inner layer of the uterus when epithelial cells lining the myometrium start to proliferate abnormally. Although most ECs are diagnosed early, mainly due to symptomatic postmenopausal metrorrhagia, up to 20% of the lesions progress to a high-stage carcinoma. Unfortunately, the five-year survival in this group of women drops to 15%, compared to 90% in women diagnosed with confined disease. Myometrial infiltration and the appearance of disseminated aggressive tumor cells are crucial events for prognosis and death in EC [2].

*Classification*

The most commonly used classification schemes are the International Federation of Gynecology and Obstetrics (FIGO) classification of grading and staging evaluated after surgical intervention. The current classification system comprises three levels based on the degree of glandular differentiation. The low grades are grades 1 and 2, where tumors show ≤5% and 6% to 50% solid, non-glandular, and non-squamous growth, respectively. The high grade (grade 3) shows more non-glandular growth, which is present in a prognostically high-risk patient group [17,18]. FIGO staging describes the spread of cancer.

In stage I, the tumor is confined to the body of the uterus, possibly extending into the myometrium. If the invasion is absent or less than half of the myometrium width is invaded, the stage is determined as

stage IA; in other cases, with greater myometrial invasion, the stage is classified as stage IB.

In stage II, the tumor spreads to the cervical stroma. Local spread outside the uterus is characteristic of stage III, reaching the uterine serosa, fallopian tubes, and ovaries in stage IIIA, the vagina or parametrium in stage IIIB, and either the pelvic lymph nodes in stage IIIC1 or the paraaortic lymph nodes in stage IIIC2.

In stage IV, cancer metastasizes to distant organs, for example, to rectal or bladder tissues in stage IVA or lymph nodes in the groin area and/or distant organs (e.g., the lungs) in stage IVB [4,5].

EC is classified into two distinct groups, type I and type II, which differ in molecular, clinical and histopathological characteristics. Type I tumors are low-grade and estrogen-related endometrioid carcinomas (EEC), while type II are non-endometrioid (NEEC), mainly serous and clear cell carcinomas. To date, this classification has been demonstrated to be an important predictor of survival, but also a determinant for the extent of the initial surgical procedure and subsequent use of adjuvant therapy. However, the molecular heterogeneity associated with the histological diversity of this type of cancer makes the current treatment options insufficiently personalized. To this regard, the integrated genomic, transcriptomic and proteomic characterization of EC performed by The Cancer Genome Atlas Research Network (TCGA) revealed four groups of tumors [3].

The first group (EEC1) includes EEC with somatic inactivating mutations in POLE exonuclease and very high mutation rates (hypermutated) (7%); it is associated with a good prognosis.

The second group (EEC2) includes EEC with microsatellite instability, frequently with MLH-1 promoter hypermethylation and high mutation rates (28%).

The third group (EEC3) is composed of EEC with low copy number alterations (39%). Importantly, both the second and third groups show similar progression-free survival rates.

The fourth group (serous-like or copy-number high) (26%) shows low mutation rate but frequent TP53 mutations. This group has worse prognosis, being predominantly composed of serous carcinomas with some sporadic cases of ECC (mainly EEC3 and some EEC1–2).

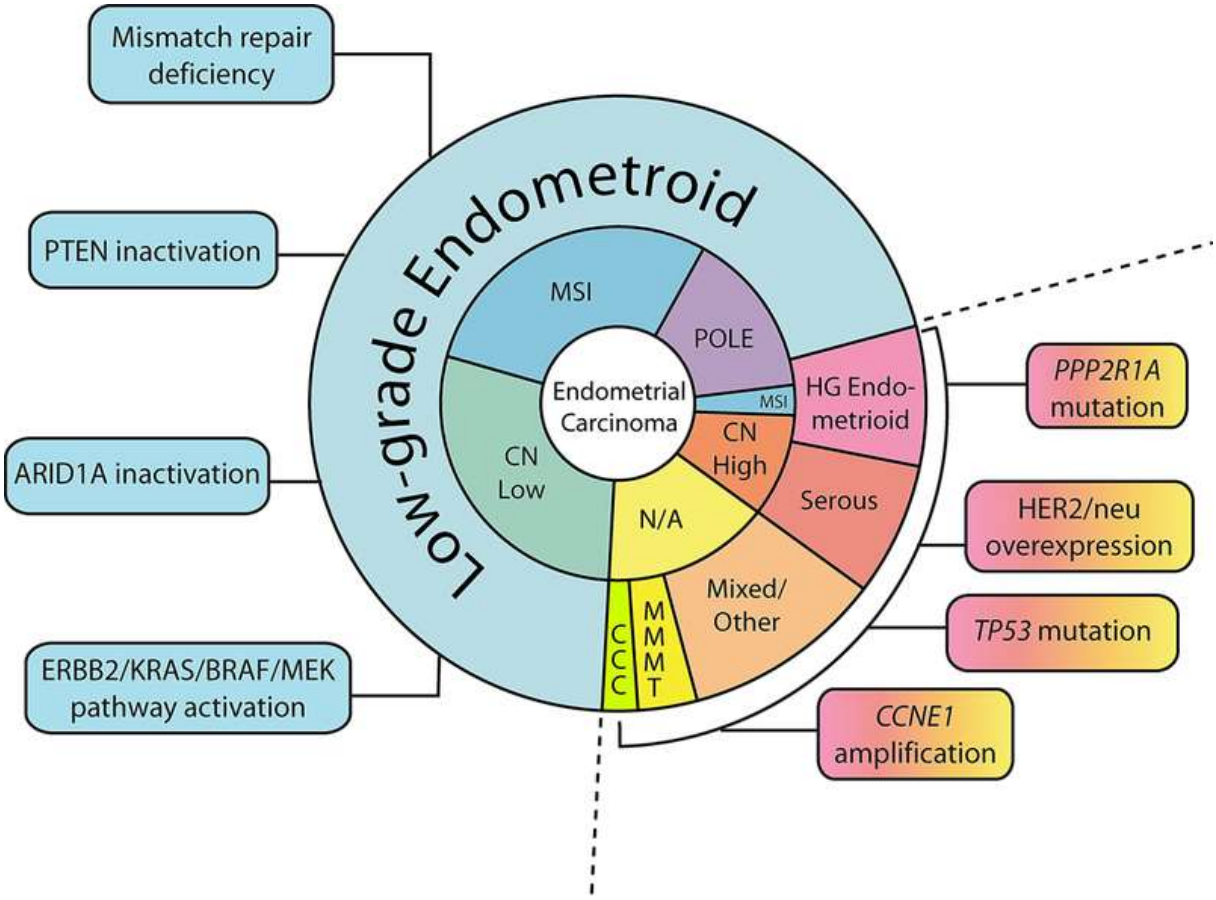


Figure 1 - **Associations of histological and TCGA classifications in endometrial cancer** [25]

As shown in figure 2 several mechanisms can lead to carcinogenesis and metastasis, including inhibiting apoptosis, inducing cell proliferation, enhancing TERT transcription, and interfering with DNA repair. *ARID1A, PTEN, KRAS, CTNNB1*, and MMR molecular pathways exclusively affect endometrioid tumor whereas serous tumors more commonly harbor alterations in *TP53, HER2, p16, CCNE1, and FBXW7*. ARID1A, AT-rich interaction domain 1A; CCNE1, cyclin E1; CTNNB1, Catenin beta-1; FBXW7, F-box/WD repeat-containing protein 7; HER2, human epidermal growth factor

receptor 2; KRAS, Kirsten rat sarcoma viral oncogene homolog; PI3K, phosphatidylinositol 3- kinase; PTEN, phosphatase and tensin homolog; TERT, Telomerase reverse transcriptase; TP53, cellular tumor antigen 53.

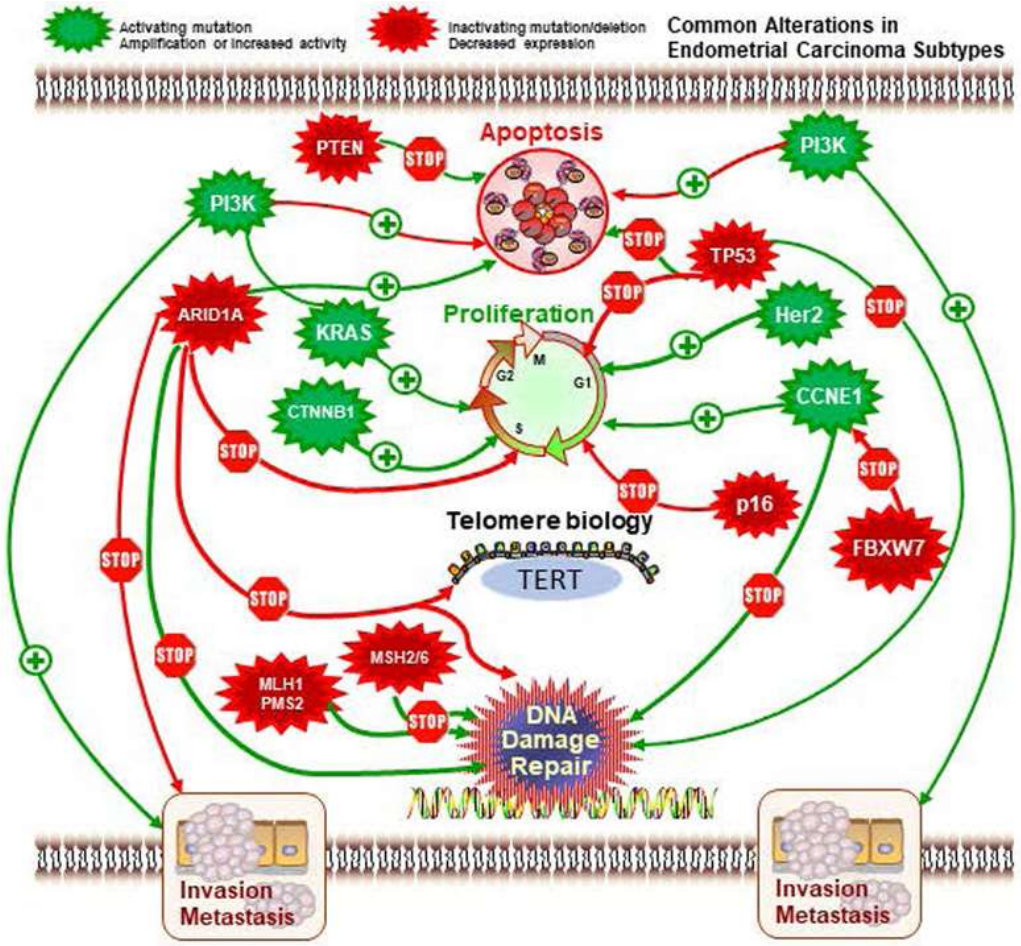


Figure 2 - **Common alterations of genes in endometrial carcinomas.**

*Diagnosis*

Approximately three-quarters of cases in postmenopausal women are diagnosed at an early stage, which improves treatment [6]. In both postmenopausal and premenopausal women, vaginal bleeding (although pathognomonic) is the most common clinical manifestation of EC [3,6]. It is recommended that all women with abnormal uterine bleeding over the age of 45 be screened for EC. Additionally, 90% of women diagnosed with EC are symptomatic. In younger women, a history of unsuppressed estrogen exposure (a major risk factor for EC) is considered [6–9]. There are no specific laboratory tests for the evaluation and screening of endometrial cancer, although transvaginal ultrasonography or endometrial biopsy are recommended, depending on the patient and the physician's preference for availability.

Symptoms suspicious for EC are post-menopausal bleeding, unscheduled bleeding on hormone replacement therapy (HRT), persistent intermenstrual or irregular bleeding, hematuria or abnormal vaginal discharge. In post-menopausal women with bleeding, transvaginal ultrasound scan (TVS) is performed as a triaging test to assess endometrial thickness (ET). If ET is > 4 mm, an endometrial biopsy is recommended as the gold standard diagnosis of EC [10]. However, in symptomatic perimenopausal or pre-menopausal women with risk factors for EC, the National Institute of Clinical Excellence recommend hysteroscopy and targeted biopsy in the first instance as TVS has limited value in women who are still

menstruating [11]. Whilst the gold standard of EC diagnosis, endometrial biopsy has an almost 99% accuracy, the current technique for triaging with ultrasound lacks specificity resulting in more than 50% of patients needing invasive biopsy [12]. Furthermore, failure to obtain a biopsy in the outpatient setting is common and occurs in around one third of women, often due to sampling failure or pain during the investigation [13]. In these patients, repeat investigations are needed, often under general anesthetic which is not without associated risks and costs [14].

*Treatment*

The most recent NCCN guidelines state that the primary treatment of endometrial endometrioid carcinoma, limited to the uterus, is a total hysterectomy, bilateral salpingo-oophorectomy and surgical staging, preferably with a minimally invasive approach when technically feasible; even young women interested in future fertility, who may benefit from fertility-sparing options, should always undergo counselling to inform them that this option is not the standard of care. In fact, they should also be encouraged to conceive as soon as the complete response is achieved and, after childbearing is completed, to then undergo radical surgery.

All of the following criteria for considering fertility-sparing options for the management of endometrial carcinoma must be met:

1. The patient must be diagnosed with well-differentiated (Grade 1) endometrioid adenocarcinoma on D&C, confirmed by expert pathology review.

2. The disease must be limited to the endometrium on MRI (preferred) or TV ultrasound.
3. There must be an absence of suspicious or metastatic disease on imaging.
4. There must be no contraindications to medical treatment or pregnancy.

Malignant carcinoma other than pure endometrioid carcinoma, such as serous carcinoma, clear cell carcinoma, undifferentiated carcinoma, and choriocarcinoma, as well as malignant mesenchymal (sarcoma), should be treated as high-grade endometrial cancer and therefore fertility-sparing surgery is not recommended [23].

*Liquid Biopsy*

Nowadays, research efforts are focused on the discovery of new non-invasive methods for the diagnosis and comprehension of the tumor molecular architecture in real time. In comparison with traditional biopsies, the study of the tumor material present in bodily fluids can provide valuable information for the diagnosis of tumors with low accessibility, or for a more complete overview of tumors in advanced stages where there are different tumor locations to be interrogated. Liquid biopsies also offer advantages to monitor the tumor evolution and the response to therapy with more accuracy than current clinical imaging techniques. In this sense, the field of liquid biopsy has emerged as a great revolution in oncology and is considered “the way” to reach precision medicine. In addition to blood, several other

bodily fluids such as saliva, urine, cerebrospinal fluid (CSF), uterine aspirates, pleural effusions or even stool have been shown high interest as a non-invasive source of tumor-derived material [15]. This tumor circulating material is mainly composed of circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating tumor miRNA, proteins and exosomes [16].

Liquid biopsy has the potential to overcome many of the limitations of the traditional biopsy-based approach in EC diagnosis, mainly due to the low cost and reproducibility of sampling [17]. Fluid collection is minimally invasive, ranging from blood, urine, lavage, cerebrospinal and peritoneal fluids to saliva samples. The analyzed samples may include circulating tumor DNA (ctDNA)/cell-free DNA (cfDNA), circulating tumor cells and proteins, and other circulating biomarkers including RNA, vesicles, and platelets [18,19]. Compared to other tumor types (breast, colorectal, cancer, and prostate), research on liquid biopsy in EC is limited and is not used in clinical practice. However, with the advent of molecular classifications, this approach shows the potential to provide diagnostic and prognostic information that could lead to screening, monitoring, and proper stratification of patients with EC [18–20]. The latest studies reveal that EC biomarkers are detectable, especially at the later stages because of the increased quantity of ctDNA/cfDNA found in blood [20,21]. 94% of patients (out of 48) in the study by Bolivar et al. have at least one EC oncomutation, mostly in the CTNNB1, KRAS, PTEN, and PIK3CA genes. However, only in one out of three of these patients was the

same mutation also present in the tumour [20]. Another, larger study (193 patients) by Danziger et al. comprised 94% of EC patients with detectable ctDNA [22].

*The ideal marker*

It is difficult to assess tissue markers without an invasive test. An ideal marker would be in blood or urine with its expression directly correlating to expression in the tissue, as well as correlating with diagnosis and prognosis of disease. A non-invasive screening marker to reduce the number of women who undergo invasive diagnostic testing is needed in endometrial cancer, as well as a prognostic marker that can aid timing of treatment for endometrial hyperplasia. A screening marker could be used at a general population level, where a positive result would determine a need for diagnostic tests. An alternative to this would be to screen the high-risk groups, such as those with Lynch syndrome and obesity only. Such biomarkers would need sufficient sensitivity and specificity, as well as being cheap and non-invasive to allow widespread use. Diagnostic biomarkers can be used either to confirm the presence of cancer or to assist in identifying a subtype and are useful for planning management, based upon grade of disease and prognosis affiliated with each biomarker [26].

## ***Materials and methods***

Eighty-five matched tumor-plasma-buffy coat samples from women diagnosed with endometrial adenocarcinoma or atypical hyperplasia from 2021–2022 at the University of Naples Federico II were included in this study.

Inclusion criteria were:

- Female sex
- Atypical Hyperplasia, endometrial cancer stage I-IV
- Age 18-80
- signing of the informed consent to participate in the study and to make available their biological samples for scientific research purposes, and to the processing of personal data.

Exclusion criteria were:

- Pregnancy
- psychiatric pathologies and/or incapacity of mind and/or will;
- Age< 18 years or over 80 years ;
- neoadiuvant chemotheraphy;
- refuse to sign the informed consent to participate in the study and to make available their biological samples for scientific research purposes, and to the processing of personal data.

Patients included in the study were chosen based on the availability of sufficient plasma, white blood cell buffy coat, and sufficient formalin-fixed, paraffin embedded endometrial carcinoma for amplicon-based next generation sequencing. Endometrial cancer

tissue and peripheral blood were both obtained at the time of hysterectomy, or at time of hysteroscopy in case of conservative management. Peripheral blood was drawn into EDTA tubes, and the plasma and buffy coat fractions were separated within 12 h of collection and stored at −80 °C.

*Liquid biopsy extraction*

To remove blood cells, blood was centrifuged at 1800× *g* for 10 min at 4 °C. Then, the supernatant was centrifuged at 16,000× *g* for 10 min at 4 °C to remove any remaining cells. Circulating tumour DNA was extracted from 2 mL of plasma, by digestion in 100 µL of proteinase K buffer for 10 min at 37 °C, followed by purification with the QIamp Circulating Nucleic Acid kit with the given protocol. The purified ctDNA was quantified by a Picogreen fluorescence assay using the provided lambada DNA standards.

*Tissue Biopsy extraction*

DNA isolation was performed through the MGF03-Genomic DNA FFPE One-Step Kit, according to the manufacturer's protocol (MagCore Diatech). DNA quality was established in triplicate using the FFPE QC Kit according to the manifacturer's protocol (Illumina, San Diego, USA). Genomic DNA was isolated from the fresh tissues using the MGF03-Genomic DNA FFPE One-Step Kit, according to the manufacturer's protocol (MagCore Diatech). DNA quantification was performed using a Qubit 3.0 Fluorometer with the Qubit dsDNA HS (High Sensitivity) Assay Kit fluorescent dye method. Following the

manufacturer instructions, we used 100 ng of DNA to perform clinical exome library preparation (Kapa HyperPlus Custom Probes, Roche).

*Lybraries preparation and Sequencing*

After the target region sequence was captured and enriched, the resulting DNA libraries were quantified with the Qubit dsDNA HS Assay Kit fluorescent dye method to determine equimolar amounts for each library. Sequencing was carried out on NovaSeq 6000 (Illumina Inc., San Diego, CA, USA) to mean sequencing depth of at least 200×. Sequence data were aligned to the human reference genome GRCh37 (http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/index.shtml (accessed on 11 October 2018)) using the Burrows-Wheeler Aligner with default parameters.

Trimming, base calling, coverage analysis, and variant calling were performed using an in house bioinformatic pipeline (bcl to fastq version 2.20, Isaac Aligner version 4, GATK “Genome Analysis Toolkit” version 4, Sam tools version 1.9 e Bed tools version 2).

Vcf analysis was performed using the GenomeUp by quality > 15, by small variant consequences such as stop gains, sand losses, splice donors, splice acceptors, splice region, frameshift indels, in frame deletions, in frame insertions, initiator codon (ATG) losses, missense protein altering, incomplete terminal codon).Additional filtering was performed at frequency < 0.05 in European populations using tools such as the 1000 Genomes Project

(https://www.internationalgenome.org/ (accessed on 11 October 2018)), gnomAD (https://gnomad.broadinstitute.org/), Exome Aggregation Consortium (http://exac.broadinstitute.org/ (accessed on 11 October 2018), and Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/ (accessed on 11 October 2018)). Variants were classified according to American College of Medical Genetics and Genomics (ACMG) guidelines [13]. We only selected variants affecting coding exons or canonical splice sites. Finally, synonymous variants were filtered out to detect only rare variants (frequency of <0.1%) in both dbSNP138 and our in-house database containing >1000 exomes) with high quality. A *in silico* panel of Endometrial tumor related genes was reported in Table 1 was used in order to search for a causative variant. Variants were classified according to American College of Medical Genetics and Genomics (ACMG) guidelines [14].

*Table 1 Endometrial tumour related genes*

| | | | |
|---|---|---|---|
| AKT1 | CSMD3 | PALB2 | MSH6 |
| APC | CSNK1G1 | PAX2 | NBN |
| ARID1A | CTCF | PDGFRA | NRAS |
| ARID1B | CTNNB1 | PDK1 | PIK3R1 |
| ATR | DRAM1 | PEX6 | POLE |
| BARD1 | EGFR | PIK3CA | PPP2R1A |
| BCL2 | ELF3 | KIT | PMS2 |
| BCOR | ENTPD5 | KLF5 | PTEN |
| BRAF | EPCAM | KMT2D | RAD50 |
| BRCA1 | ERBB2 | KMT2C | RAD51C |
| BRCA2 | ERBB3 | KRAS | RAD51D |
| BRIP1 | FANCM | LAMB1 | RB1 |
| CCNB2 | FAT1 | LIN54 | RNF43 |
| CDH1 | FAT3 | LRP1B | SMARCA4 |
| CDKN2A | FAT4 | MAPK1 | SMARCB1 |
| CORO2A | FBXW7 | MAP2K6 | SPOP |
| | FGFR2 | MED12 | STK11 |
| | FOXL2 | MET | TERT |
| | FRMD8 | MLH1 | TP53 |
| | HNF1A | MRE11 | ZFHX3 |
| | HRAS | MSH2 | |
| | IMP3 | | |
| | KIAA1324 | | |

## ***Results***

Eighty-five patients were primally enrolled in the study, but 8 were excluded for lack of inclusion criteria.

The histological characteristics of the sample collected are summarized in table 2.

*Table 2 Histology*

| | N= | Percentage (%) |
|---|---|---|
| **Type 1 endometrioid** | 43 | 55 |
| **Atypical hyperplasia** | 30 | 38 |
| **Sarcoma** | 2 | 2,5 |
| **Type 2** | 2 | 2,5 |
| | 77 | 100 |

Of the 77 samples included in the study, 43/77 had histological diagnosis of type 1 endometrioid carcinoma, 30/77 of atypical hyperplasia, 2/77 of sarcoma, 2/77 of type 2 endometrial carcinoma.

All patients with diagnosis of endometrial cancer underwent demolitive surgery, while 10/30 patients with diagnosis of atypical hyperplasia in childbearing age (mean age 30,6 ± 5,4) decided for conservative treatment.

Clinical and pathological characteristics of enrolled patients were summarized in table below (table 3-5 )

Table 3 - Mean age

| Histology | Mean | N | Dev. std. |
|---|---|---|---|
| Type 1 | 61,775 | 43 | 10,5672 |
| Atypical Hyperplasia | 47,417 | 30 | 12,9040 |
| Sarcoma | 44,500 | 2 | 3,5355 |
| type 2 | 74,000 | 2 | 4,2426 |

Table 4 – atypical hyperplasia, treatment choice

| | | N | Mean | Deviazione std. | |
|---|---|---|---|---|---|
| | Conservative | 10 | 30,600 | 5,4589 | 2,4413 |
| | Demolitive | 20 | 56,429 | 9,5391 | 1,8027 |

Table 5 – Tumor characteristics

| | | | |
|---|---|---|---|
| GRADING | G1 32% (14/43) | G2 39% (17/43) | G3 28% (12/43) |
| MIOMETRIAL INVASION | <50%<br>65% (28/43) | >50%<br>35% (15/43) | |
| LINPHOVASCULAR INVASION | NO<br>64% (27/43) | YES<br>36% (16/43) | |
| TYPE OF SURGERY | LAPAROSCOPY<br>9% (6/66) | LAPAROTOMY<br>27% (18/66) | ROBOTIC LAPAROSCOPY<br>60% (40/66) |

The mean cell-free DNA yield extracted from the plasma samples was 18.6 ng/ml (range, 6–30 ng/ml).

At least one pathogenic mutation in plasma samples was detected in 70/77 (94%) of the tumors analyzed (chi square 28; p=0,01), the detected mutation on plasma was the same of the one detected in solid tumor in 50/77 (65%) of cases.

The concordance was higher for atypical hyperplasia (75%) than type 1-2 endometrial cancer (55%).

The cfDNA expression in plasma do not relate with grading (p>0,05) but is associated with myometrial infiltration (chi-square 25; p=0,001).

The pathogenic mutation identified in plasma or in solid tumor was found in the germinal line in 19% of endometrioid cancer and in 14% of atypical hyperplasia. In 42% of endometrioid cancer and 28% of atypical hyperplasia the gene in the germinal line had a benign

mutation but acquired in plasma or in solid tumor a pathogenic mutation. In 38% of endometroid cancer and 21% of atypical hyperplasia the mutation of the gene was not present in the germinal line (chi-square 26; p=0,03).

In the sample included in the study the gene that were found more often mutated are ATR, BRAF, CTCF, CTNNB1, FGRFR2, KMT2D, KMT2C, MSH, PIK3RI, PTEN, ZFHX3. The concordance in the mutation between the solid tumor and the plasma was principally found in KMT2C, PIK3RI, PTEN and ZFHX3.

The presence of cfDNA mutation of PTEN (chi-square 25; p<0,001) and PIK3RI (chi square 22; p<0,001) in plasma related with grading G3 and >50% infiltration. The presence of cfDNA mutation of CTCF (chi-square 12; p<0,01) and BRAF (chi square 18; p<0,001) related with >50% infiltration.

ZFHX3, P53, PTEN was associated with diagnosis of endometrioid cancer while KMT2C was associated with atypical hyperplasia.

Discussion

A total of 94% of patients with EC/AH had detectable ctDNA, according with other studies [20-22] and 65% had a potentially targetable alteration or biomarker, confirming liquid biopsy as a viable alternative in EC when appropriate tumor tissue is lacking and could increase access to clinical trials.

Patients normally have good prognosis if diagnosis occurs in early stages of EC. However, some patients experience recurrence after

surgery, and this recurrence is not predictable with the current risk classification systems. Clinical management of the risk of recurrence remains an unsolved issue that is probably associated with tumor heterogeneity and early tumor dissemination [27]. The advantages of using liquid biopsies rather than tissue samples are clear: the samples are easy to obtain, provide information in real time, and improve the understanding of tumor heterogeneity.

In our study we compare the cfDNA mutazion on plasma with mutations identified in solid tumor. Those data were compared with the analysis of germinal line of the same patients to identify de novo mutations. We were able to detect genetic alterations in 93% of the UA samples analyzed with targeted sequencing ATR, BRAF, CTCF, CTNNB1, FGRFR2, KMT2D, KMT2C, MSH, PIK3RI, PTEN, ZFHX3 were the genes most frequently mutated in our cohort, in accordance with the genomic pattern previously described in primary carcinomas [3,28,29,30]. The presence of cfDNA mutation of PTEN is usually associated with PIK3RI [3] and in our cohort related with grading G3 and >50% infiltration. Moreover, the rate of ctDNA-positive cases found correlated with myometrial and lymphovascular infiltration and with histology grade, in line with recent data [20].

Another important data is that pathogenic mutation identified in plasma or in solid tumor was found in the germinal line in 19% of endometrioid cancer and in 14% of atypical hyperplasia. In 42% of endometrioid cancer and 28% of atypical hyperplasia the gene in the germinal line had a benign mutation but acquired in plasma or in solid

tumor a pathogenic mutation. This could led to the identifications of patterns of genetic predisposition to the cancer.

Long-term follow-up of the patients included in the study will be necessary to confirm the clinical value of ctDNA determination in improving the risk classification of patients with EC. In this regard, Pereira et al., by analyzing a retrospective cohort of gynecologic tumors (ovary and endometrial tumors), have found lower survival rates in patients with detectable ctDNA levels at surgery [31].

No matter the specific technique employed, the liquid biopsy approach has long been touted as a means of early cancer diagnosis or a way to perform cancer screening in at-risk individuals in a relatively non-invasive way. The use of liquid biopsy for this clinical application has pitfalls, however. It is known that somatic mutations in important driver genes are not restricted to malignancies. For example, it was recently demonstrated that 79% (19/24) of women with endometriosis had mutations in genes such as ARID1A, KRAS, and PIK3CA in the epithelial component of the endometriotic lesion tested [32].

A more conservative, but still useful, clinical application for liquid biopsy in endometrial cancer could be as a non-invasive method of monitoring a patient for recurrence following hysterectomy or for patients who underwent conservative management of atypical hyperplasia. In this scenario, it would be necessary to first characterize the molecular changes in the primary tumor, as any mutations identified in the tumor could then be tracked longitudinally

in blood samples. From the current study, using a next-generation sequencing panel of only four genes, more than 90% of patients with endometrioid-type endometrial carcinoma have at least one mutation identified and could be potentially followed with serial liquid biopsies. Prospective studies are necessary to determine whether plasma-based mutations in these genes could be detected years after the hysterectomy and whether detection of such plasma mutations is an early indicator of tumor recurrence.

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