Abstract P323

The HPV RNA assay as follow-up test

M. d'Avenia*[1], M Ciarmoli[1], M. E. Ferraro[1] , C. Malta[1], C. Violante[1], M. De Santis[1] , M. Russo[1] , L. Santomauro[1], S. Stella[1], A. Maglietta[1], M. Mastromauro[1], E. Adorisio[2], M. Iacobellis°[1].

[1]UOSVD Cytopathology & Screening PO Di Venere ASL BARI

[2]UOC Clinical Pathology, Dept. Laboratory Medicine, PO Di venere ASL BARI

*morena.davenia@asl.bari.it ° michela.iacobellis@asl.bari.it

Introduction

Among the IVDR tests for the diagnosis of the Human Papillomavirus (HPV) infection, the ones validated for the screening of the cervical cancer target all HPV DNA. They are mainly based on PCR or on the Hybrid Capture or Invader technologies. There are also diagnostic tests, based on the detection of HPV RNA, not yet validated for the screening. They target the transcripts of the E6 and E7 viral oncogenes, which are sequences integrated into the host genome and expressed (Fig. 1). Since women with a Pap test positive for high-grade lesions and with a CIN2+ histology may result negative to screening tests targeting L1-DNA of high-risk (HR) strains, the aim of this work was to evaluate the HPV-RNA test by studying the host infection phase (Fig. 2 and 3).

Methods

In the first quarter of '22, 1074 cervical samples in PreserveCyt (females 25-64 y.o.) were tested with both Cobas 4800 HPV Real Time PCR (Roche) - L1 DNA gene target, and Aptima HPV TMA Test on Panther platform (Hologic) - E6-E7 mRNA targets. The results were correlated with the Pap tests and, when possible, with the post-colposcopy biopsies received in the following 6 months. All the samples with discordant DNA/RNA results and those resulted negative to both tests but positive at the histology were genotyped with AnyplexII HPV28 det (Seegene) and with Neoplex HPV29 (Eurospital).

Results

HPV DNA/RNA co-testing, Pap Test and/or histological results were the following:

292 DNA+/RNA+ cases: PCR HR-HPV Ct_m27.9±5.6, IC Ct_m 27.5±2.1. TMA HR-HPV RLU_m1527224±899000. Cytology: 161 ASC-US, 68 L-SIL, 14 ASC-H, 47 H-SIL, 2 Adenocarcinomas in situ (AIS). 97 biopsies received of which 58 with CIN2+ histology.

34 DNA-/RNA+: PCR HR-HPV Ct_m n/a, IC Ct_m 27.5±2.1. TMA HR-HPV RLU_m619000±499000. Aptima identified 14 cases with HR genotypes (HPV58-66-68), 10 Low-Risk (LR) of which 9 putative HR (pHR), 10 negative cases also to confirmatory tests. Cytology: 3 negatives, 8 ASC-US, 17 L-SIL, 4 H-SIL, 2 ASC-H. 14 biopsies received, 7 CIN2+ (1 HPV 58, 2 HPV68, 3HPV 82, 1HPV 67, specifically detected by the Neoplex kit).

76 DNA+/RNA-: PCR HR-HPV Ct_m35.3±3.6, IC Ct_m 27.6±1.9. TMA HR-HPV RLU_m: 2356±1252. In particular, Aptima did not detect 7 positive cases for HPV16 and 4 HPV18, 6 cases were negative also to confirmatory tests, the remaining were positive for the other HR genotypes. Cytology: 12 negatives, 24 ASC-US, 35 L-SIL, 2 ASC-H, 1 H-SIL. 43 biopsies were received, 2 CIN2+ (HPV31).

672 DNA-/RNA-: PCR HR-HPV Ct_m n/a, IC Ct_m 28.77±2.5. TMA HR-HPV RLU_m:2696±1980. Cytology: 72 negatives, 526 ASC-US, 58 L-SIL, 6 H-SIL, 8 ASC-H, 1 AGC, 1 AIS. 115 biopsies were received, 9 CIN2+ (2HPV53, 1HPV44, 2HPV73, 1HPV82, 2 negatives even with confirmatory tests, AIS was negative with all assays, probably due to sample collection problems, in fact a previous sample from the same patient was HPV31 positive. Tab. 1, 2 and 3.

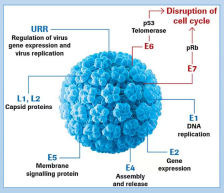


Figure 1. Two of the early HPV genes encode for the oncoproteins E6 and E7. mRNA transcription in cells indicates active viral processes. Overexpression of E6 and E7 mRNA precedes transformation of cells. In persistent high risk HPV infections, E6 and E7 oncoproteins disrupt the p53 and retinoblastoma protein (pRb) cell cycle control mechanisms respectively. *https://diagnostics.roche.com*

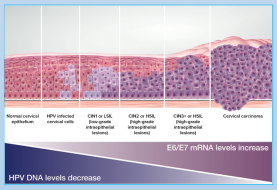


Figure 2. mRNA identifies the presence and activity of a high-risk HPV infection. E6/E7 mRNA expression is indicative of the HPV infections most likely to lead to disease. In fact, HPV DNA tests only identify the presence of any of the 14 high-risk HPV types, but not the oncogenic activity. *https://hologicwomenshealth.com/*

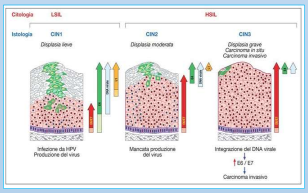


Figure 3. in early phases of infection, high virus production occurs and huge amounts of viral DNA copies are synthetized, leading to feasible Viral DNA detection; Cervical cancer represents a "dead end" for the virus, because viral DNA becomes integrated into the host cell chromosome (often with large deletions), and the ability to produce infectious virions is abolished. This happens more commonly in cervical lesions caused by high-risk papillomavirus types such as HPV16. Red circles, cells expressing E7; dark blue circles, nuclei of uninfected cells. Cells expressing E4 are depicted as green. Nuclei of cells expressing L1 are shown in orange, whereas those that contain only amplified viral DNA are turquoise. Middleton et al. *Journal Of Virology*, 2003: 10186–10201

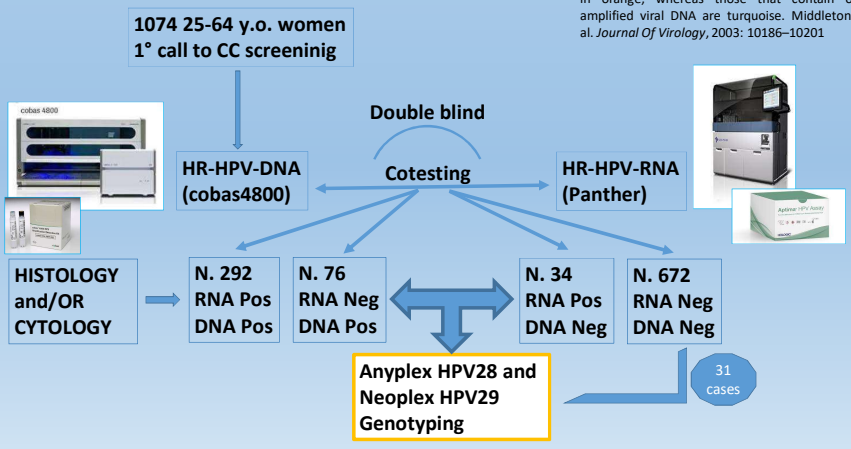
Tab.1		DNA L1 target	
		+	-
E6/E7 RNA target	+	292	34[a]
	-	76[b]	672[c]

Tab.2		Disease	
		+	-
E6/E7 RNA target	+	299[d]	27[e]
	-	11[f]	737[g]

Tab 1 and 2. HPV RNA assay performance vs DNA test and vs preliminary clinical outcome or infection evidence:

a. 14/34 cases resulted positive to HR HPV genotypes, even if DNA test was negative (3 HPV58, 9 HPV68, 1 HPV66, 1 HPV56), the others were negatives (10) or LR (10). 7 CIN2+ (1 HPV 58, 2 HPV68, 3HPV 82, 1HPV 67, specifically detected by the Neoplex kit).
b. 7/76 resulted negatives to genotyping. The other were positives (TOCE ®+ or Real Time PCR late Ct) to HR genotypes (7 HPV16, 4 HPV18, 8 HPV31 of which 2CIN2+, 3 HPV33, 1 HPV35, 5 HPV39, 5 HPV45, 11 HPV 51, 5 HPV52, 5 HPV56, 5 HPV58, 7 HPV59, 11 HPV66, 3 HPV68, in single or multiple infection (n.7))
c. 9 Cin2+, 8 not HR-HPV related, 1 HPV31+ not detected due to pre-analytical problems).
d. 299 cases positives with both the assays + 7CIN2+ RNA+/DNA- *
e. Cases RNA+/DNA- without a Cin2+ diagnosis
f. 9 cases DNA-/RNA- Cin2+ and 2 cases DNA+/RNA- Cin2+
g. 663 cases DNA-/RNA- and 74 cases DNA+/RNA- with negative cytology, colposcopy or histology <Cin2 *

*(final disease outcome will be available at 3 years follow up; results concordance has been assumed as confirmation or absence of infection).



Tab. 3 Target Amplification signals	HPV DNA PCR Ct (mean ± ds)	IC (b- globin DNA) PCR ct (mean ± ds)	HPV mRNA target TMA RLU (mean ± ds)
DNA+/RNA+	27.9±5.6	27.5±2.1	$1,52x10^{\wedge 6}$±$8,9x10^{\wedge 5}$
DNA-/RNA+	n/a	27.5±2.1	$6,19x10^{\wedge 5}$±$4,9x10^{\wedge 5}$
DNA+/RNA-	35.3±3.6*	27.6±1.9	$2,3x10^{\wedge 3}$±$1,210^{\wedge 3}$
DNA-/RNA-	n/a	28.77±2.5	$2,6x10^{\wedge 3}$±$1,9x10^{\wedge 3}$

Tab. 3 HPV targets detection cycle threshold (Ct) in Real Time Polymerase chain reaction (PCR) or Relative Luminescence Units (RLU) in Transcription mediated Amplification (TMA). * DNA+/RNA- samples were significantly detectable at late cycles in PCR, indicating Low copies number in PCR reaction, due to Low Viral Load or poor sample collection. P <0,01

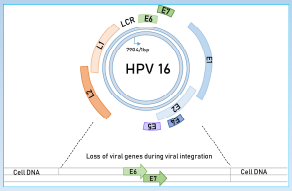


Figure.4. Upon integration, most of the regulatory genes (E1, E2, E4, and E5) and the capsid genes (L1 and L2) are lost, but two main oncogenes, E6 and E7, remain uncontrollably expressed and are no longer under negative control of the E2 protein. This gives the cells integrated HPV genomes a selective growth advantage and promotes the development of cancer. Pesut *et al. Viruses* **2021**, *13*(11), 2234.

Conclusions

Even if the HPV-RNA assay may cross-reacts with non-HR genotypes, it may be very useful in the detection of high-grade lesions related to pHR HPV strains, in the identification of patients with the integrated virus genome, rid of the L1 region (Fig.4) or in pseudo-latency phase and in cancer risk-stratification. In fact, preliminary clinical sensitivity and specificity of HPV-RNA assay were 96.4% and 96.5% vs 94,8% and 90,3% of HPV-DNA assay.