oncgnostics
MOLECULAR CANCER DIAGNOSTICS

Summary

of all presentations at
EUROGIN congresses **2018-2024**


GynTect®
Diagnostic kit for the qualitative detection of epigenetic markers in bisulfite-converted DNA from cervical samples.
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GynTect®
for LBC samples
REF GT002-10
LOT Z113
2018-10-11
IFU Version: Rev 2.5
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oncgnostics presenters

Dr. Alfred Hansel
Biologist
CEO oncgnostics GmbH

Kristin Knoll
Pharma-Biotechnologist
oncgnostics GmbH

Dr. Martina Schmitz
Biochemist
CSO oncgnostics GmbH

Anna-Bawany Hums
Molecular Biologist
oncgnostics GmbH

Dr. Carolin Dippmann
Pharma-Biotechnologist
oncgnostics GmbH

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A panel of six DNA methylation markers, comprising the GynTect cervical cancer triage, display excellent sensitivity for cervical carcinomas*

Presenter: Dr. Alfred Hansel
Biologist | CEO oncgnostics GmbH | 2018

Objectives

A prerequisite for triage tests complementing HPV-based cervical cancer screening, which now is or is being established in several countries, is to detect cervical cancer with high sensitivity at no loss of specificity. DNA methylation may provide an attractive option in that context, and with GynTect a molecular diagnostic test based on this class of markers is available. The aim of this study was to assess the methylation of the six markers comprising GynTect in CIN3, carcinomata in situ, and invasive cervical carcinomas using both, cervical tissue and cervical scrapes.

Methods

DNA isolated from 155 cervical cancer tissues as well as scrapes from 121 patients with either carcinoma in situ or cancer, taken before surgical treatment at the university women's hospital in Jena, were included in the study. Methylation of the six marker regions ASTN1, DLX1, ITGA4, RXFP3, SOX17, and ZNF671, altogether comprising the GynTect® diagnostic test, was assessed performing methylation-specific PCR on chemically treated DNA from the samples.

Results

All 155 cervical tissues studied showed methylation of at least two of the six markers, and each of the methylation markers was positive in at least 80% of the cervical cancer tissues. Only one of 49 non-cancer tissues was positive (Fig. A). Similar results were obtained when assessing the methylation of the six markers in cervical scrapes: 67 of 69 scrapes from cancer patients were marker-positive (Fig. B). With the CE IVD-marked GynTect assay used on cervical scrapes, sensitivity for cancer cases was improved: all 36 cancer cases were GynTect-positive, and with 152 of 282 CIN3 cases being positive the sensitivity for these precancerous lesions remained high. Interestingly, the GynTect detection rate (14 of 16 cases) was much higher in "carcinoma in situ" (Fig. C)

Conclusion

The six DNA methylation markers can be detected in tissue as well as scrapes obtained from cervical cancer patients. The GynTect assay for cervical cancer triage that is based on these six markers, allows detection of cancer cases from cervical scrapes with 100% sensitivity.

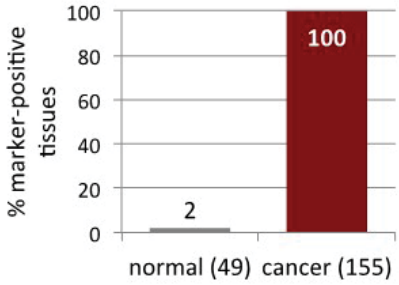


Figure A: Marker validation in tissue

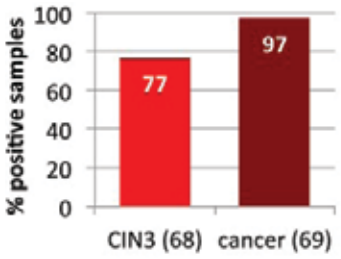


Figure B: Methylation marker validation in cervical scrapes

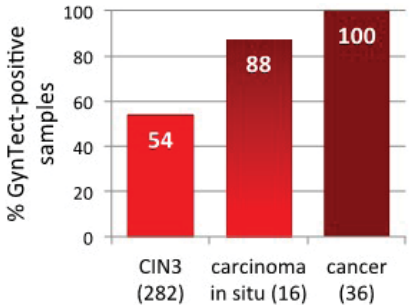
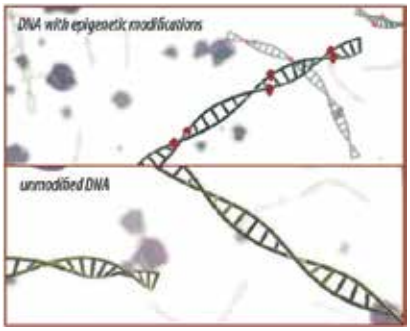


Figure C: GynTect® positivity rate in cervical scrapes from patients with CIN3, carcinoma in situ and cancer.

* Hansel A.[1], Schmitz M.[1], Dürst M.[2]
[1]oncgnostics GmbH, Jena Germany;
[2]Department of Gynecology, Jena University Hospital, Jena, Germany

Six methylation markers, known as GynTect assay, show a very good performance in a triage setting on HPV positive women*

Presenter: Dr. Martina Schmitz
Biochemist | CSO oncgnostics GmbH | 2018



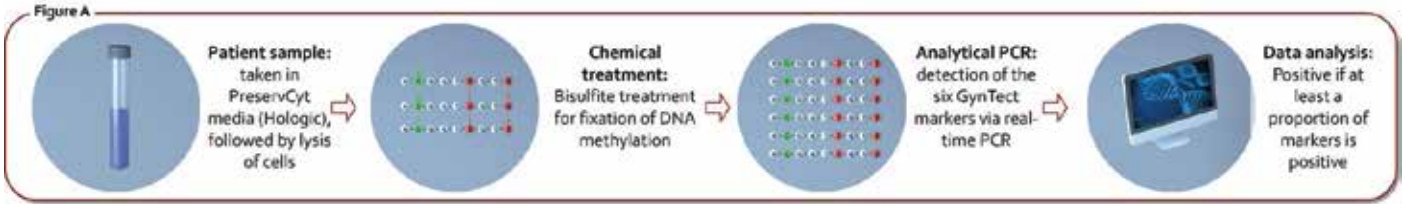
Objectives

HPV DNA testing as a primary screening marker is being implemented in several countries. Due to the high HPV prevalence in the screening population, effective triage strategies for HPV-positive cases are required. The aim of this study was to evaluate the performance of a methylation-specific real-time PCR assay (GynTect) comprising six marker regions as a triage test.

Methods

In a retrospective, cross-sectional study with the colposcopy clinic of Jena University Hospital, cervical scrapes from 675 patients were analyzed using methylation specific PCR for 6 promising DNA methylation marker regions (ASTN1, DLX1, ITGA4, RXFP3, SOX17, ZNF671), the so called GynTect markers. GynTect workflow is shown in Figure A. We correlated the GynTect results to histopathology findings for all HPV-positive samples. Samples from completely unsuspicious patients (Pap I and HPV negative) do not have a histopathology correlate.

Figure A



Results

The GynTect methylation markers show a 100% sensitivity for CIS and cancer scrapes (n=31), irrespective of subtype. 64.1% CIN3 were detected followed by

Table A

	HPV-Status	Samples in total	GynTect positive	Detection rate %
healthy	neg	305	25	8.2 %
Biopsy taken: no CIN	pos	185	46	24.9 %
Biopsy taken: undefined suspicious	pos	33	19	57.6 %
CIN 1	pos	8	1	12.5 %
CIN 2	pos	10	3	30.0 %
CIN 3	pos	103	66	64.1 %
ACIS	pos	2	2	100.0 %
CIS	pos	7	7	100.0 %
Invasive Cancer	pos	22	22	100.0 %

* Schmitz M.[1], Wunsch K.[1], Hippe, J.[1], Greinke C.[2], Dürst M.[2], Hansel A.[1]
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30% CIN2 and 12.5% CIN1, respectively. In the HPV-positive, but biopsy-proven "no CIN group", 25.9% were tested GynTect-positive. The healthy group had a positivity rate of 8.2% (all shown in Table A and Figure B). In total, sensitivity and specificity for CIN3+ in this cohort was 72.4% and 85.2%, respectively (Table B).

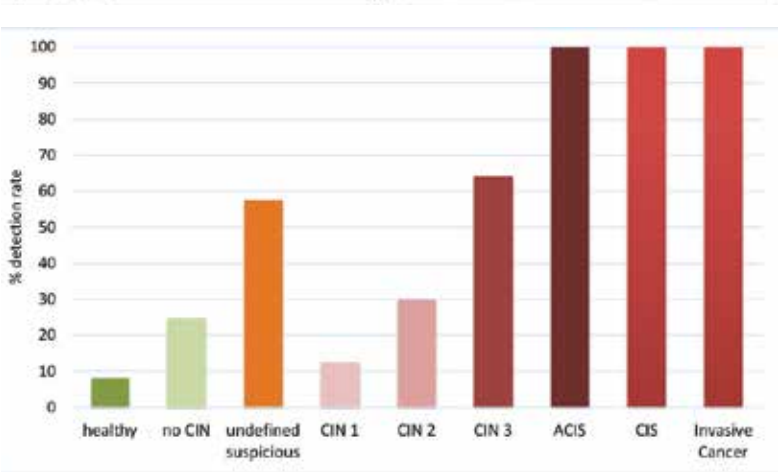

Figure B

	Sensitivity	Specificity	Prevalence
CIN2+	69.4%	85.5%	22.4%
CIN3+	72.4%	85.2%	20.9%

Table B

Conclusion

The performance of the GynTect assay on cervical scrapes from the colposcopy clinic in Jena provides good evidence for the usefulness of methylation markers to detect HPV-positive women with clinically relevant disease. Ongoing studies aim to show the prognostic potential of a negative GynTect result, an essential proof that all CIN2 and CIN3 not detected with GynTect will not develop a cancerous disease.

Epigenetic markers allowing for early risk determination for cervical neoplasia and cancer*

Presenter. Dr. Alfred Hansel
Biologist | CEO oncgnostics GmbH

Objectives

Cervical cancer develops slowly from lesions, so-called cervical intraepithelial neoplasias (CIN) as a consequence of persisting HPV infection. Neither at the primary HPV infection stage, nor at the manifestation of the different premalignant lesions current cervical cancer screening methods allow to distinguish between infections and lesions that will clear and those that may persist and develop into cancer. Markers with prognostic potential would allow for successful treatment of those lesions that may develop into cancer, at an early stage, which increases the chances for full cure. Whereas women with lesions

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that have the potential to regress, will profit from such a test, as they will not undergo long-term watchful waiting and eventual unnecessary treatment.

Methods

In a retrospective, longitudinal study cervical scrapes from 30 patients with final histopathologically assured diagnosis CIN3, for whom samples from visits even before the diagnosis CIN3 were available, were analysed for methylation of the three markers contained in the GynTect® test for cervical cancer diagnostics, ASTN1, DLX1, and ZNF671. The methylation status of the three markers was determined using methylation-specific PCR and correlated to histopathological and cytological findings.

Results

In the small longitudinal study comprising 30 patients detection (up to six years) of the markers ZNF671, DLX1, and ASTN1 was obtained in 50%, 40% and 30% of all cases at a time point where no histopathological signs of a lesion were determined. In some of these cases the markers were detected more than 2 years before CIN3 was diagnosed. In a control group comprising 552 patient samples with Pap I findings, the detection rate was significantly lower with 0.9%, 11.1% and 3.6%, respectively.

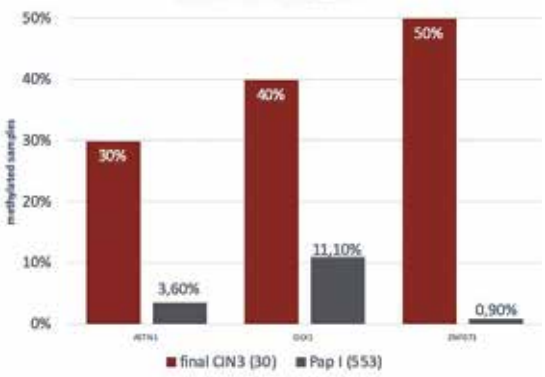


Fig. 1: Methylation of the markers ASTN1, DLX1, ZNF671 in 30 patients with later diagnosis CIN3, compared to women with no abnormal cytology finding (Pap I, 553 patients).

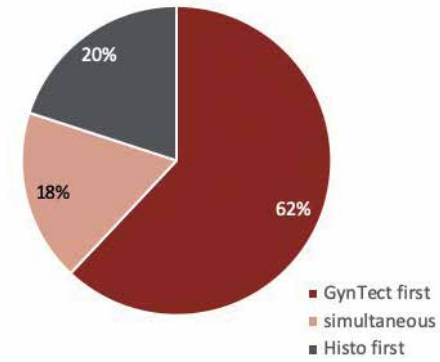


Fig. 2A: GynTect marker detection among 119 women with final histopathology HSIL/CIN3.

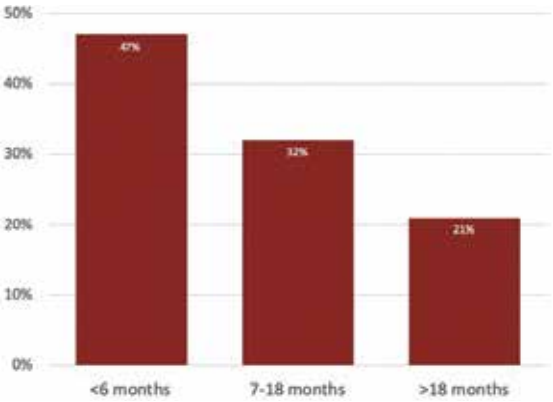


Fig. 2B: Time of earlier detection in those 62% of cases where GynTect markers were detected before the CIN3 hsitopathology finding..

Conclusion

The results of this study underscore the prognostic value of the markers for severe cervical dysplasia. With the prospective trial GynTect-PRO we aim to confirm the prognostic value of all six GynTect® methylation markers.

Analysis of diagnostically relevant DNA methylation marker regions in cervical cancer and its precancerous lesions using next generation sequencing*

Presenter: Dr. Carolin Dippmann
Pharma-Biotechnologist | oncgnostics GmbH

Objectives

DNA methylation, as an epigenetic mechanism, is an early event in cervical carcinogenesis. In several studies, indications were found that the degree of methylation of marker regions may correlate with the severity of the lesion. The objective of this work is to explicitly assign the methylation of diagnostically important marker regions to tumour cells. Furthermore, the methylation level of these marker regions is investigated in relation to the severity of the lesion.

Methods

DNA recovered from manually microdissected fresh-frozen CIN and cancer tissue (CIN/tumour as well as stroma regions) was bisulfite-treated and subjected to a subsequent bisulfite-specific PCR. PCR amplicons obtained for the marker regions ASTN1, DLX1, ITGA4, RXFP3, SOX17, ZNF671, FAM19A4, mir124-2 and POU4F3 were sequenced for each sample on an IonTorrent PGM using the Ion 318 Chip Kit. The reads were mapped against the human reference genome hg38. In total, 56 samples from 41 patients are being examined. The samples were histologically defined as 6 CIN1, 9 CIN2, 16 CIN3 and 25 cervical cancer samples.

Results

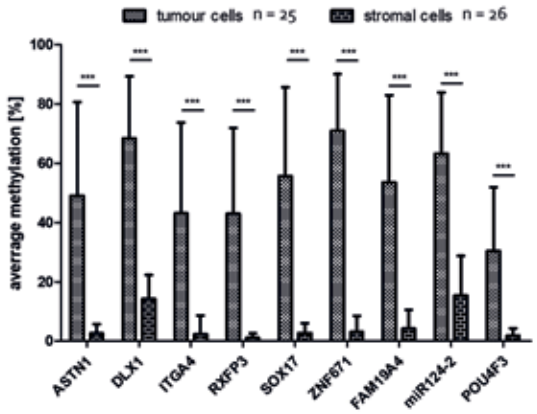


Figure A: Average methylation level of diagnostically relevant DNA marker regions

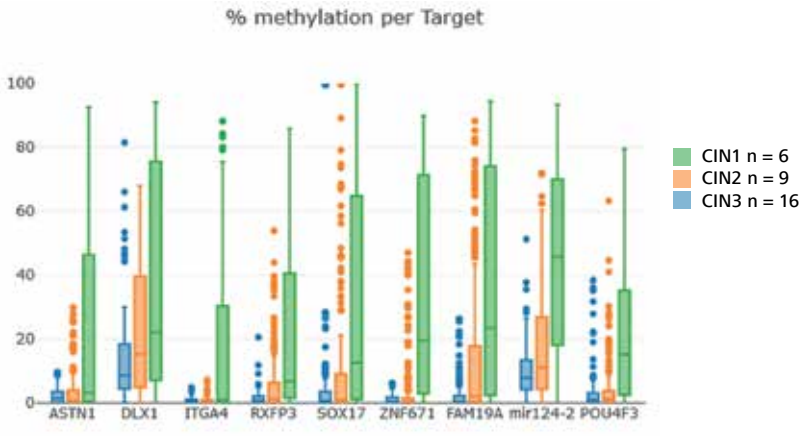


Figure B: Average methylation level of precancerous lesions

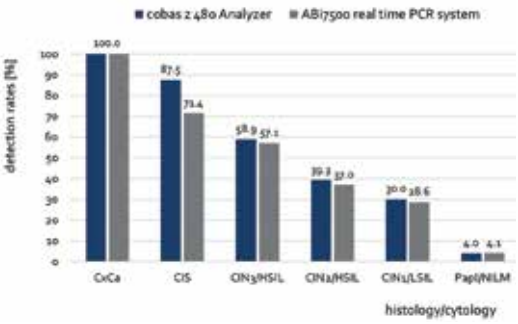
*Carolin Dippmann[1], Martina Schmitz[1], Alfred Hansel[1], Julie Krainer[2], Klemens Vierlinger[2], Lars Jansen[3], Matthias Dürst[3]
[1]oncgnostics GmbH, Jena, Germany; [2]Austrian Institute of Technology GmbH, Vienna, Austria,
[3]Department of Gynecology, Jena University Hospital, Jena, Germany

Conclusion

In general, the average methylation is higher than 40% for tumour cells and lower than five percent for stromal cells. Due to the significant difference in the methylation level between tumour and stromal cells, a clear assignment of methylation to the tumour regions can be made. In a first overview it seems that the methylation of the markers increases with the severity of the lesion.

Performance of GynTect® – a DNA methylation marker panel-based diagnostic test, on a widely used diagnostic platform

Presenter: Kristin Knoll
Pharma-Biotechnologist | oncgnostics GmbH



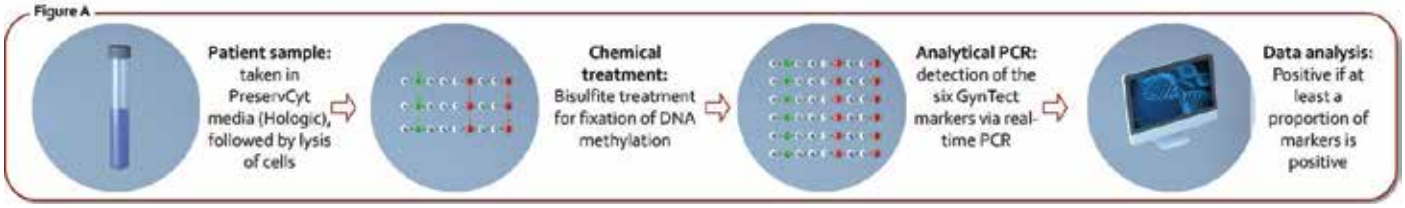
Objectives

A change of the current screening algorithms to an HPV-based screening setting is discussed in several countries due to higher sensitivity of HPV testing compared to cytology. Reliable triage methods, ideally performed from the sample obtained for screening are, however, essential in such a setting to avoid overtreatment and higher screening costs. Specific DNA methylation patterns may provide a suitable triage tool, and they can be detected using molecular tests that do not require specific equipment.

Methods

Cervical scrapes collected in PreservCyt® solution (Hologic) from women with cervical cancer (13 cases), carcinoma in situ (9 cases), CIN1-3 (106 cases) and normal cytology (Pap I; 200 cases) were assessed for methylation of the marker regions ASTN1, DLX1, ITGA4, RXFP3, SOX17, and ZNF671 (GynTect assay) comparing the ABi7500 real time PCR system (Life Technologies) routinely used for the assay with the cobas z 480 Analyzer (Roche Diagnostics).

Figure A



Results

100% sensitivity for cancer cases could be confirmed (Figure 1) and a specificity for CIN3+ of 90% was achieved on both PCR systems (Table 1). The false-positive rate among PapI samples was 4.0% and 4.1%, with the cobas z 480 and the ABi7500 system, respectively. Overall, the concordance between the two PCR systems in this sample setting was 95.5%. Furthermore, in a repeatability trial with 60 samples, 95% (cobas z 480) and 98% (ABi7500), respectively, of consistent GynTect results were obtained.

Conclusion

The newly established PCR protocol for the cobas z 480 Analyzer allow the performance of the GynTect assay with an accuracy comparable to that achieved with the ABi7500 real time PCR system. Irrespective of the PCR system used, the study indicates a 65% chance of having a CIN3 or cervical cancer at a positive GynTect test result. A negative GynTect test result excludes the presence of CIN3 or cervical cancer with 90% probability.

CIN3+	cobas z 480 Analyzer	ABi7500 real time PCR system
Sensitivity	66.7%	63.4%
Specificity	89.9%	90.1%
PPV	65.8%	65.2%
NPV	90.1%	89.4%

Comparison of two methylation-based diagnostic assays on a cohort of 140 HPV-positive cervical scrapes: GynTect® and QIAsure Methylation Test*

Presenter: Dr. Carolin Dippmann
Pharma-Biotechnologist | oncgnostics GmbH

Objectives

HPV DNA testing as a primary screening marker is being implemented in several countries. Although highly sensitive, HPV testing has only limited specificity. Due to the high HPV prevalence in the screening population, effective triage strategies for HPV-positive cases are required. Epigenetic biomarkers are presently discussed as a suitable tool for triaging HPV-positive women. We compared the two methylation-based diagnostic assays GynTect and QIAsure Methylation Test.

Methods

140 HPV-positive cervical scrapes from the colposcopy clinic of the university hospital in Jena were assessed for methylation of the marker regions ASTN1, DLX1, ITGA4, RXFP3, SOX17 and ZNF671 comprising the GynTect test and for methylation of the marker regions FAM19A4 and hsa-mir124-2 comprising the QIAsure Methylation Test. (Experiments-IFU compliant; see figure A for procedure of GynTect and QIAsure Methylation Test)

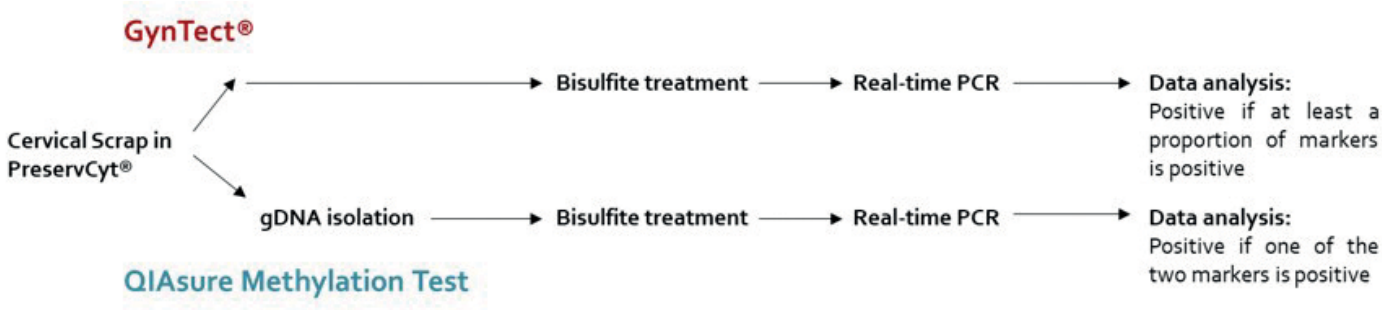


Figure A: Procedure of GynTect® and QIAsure Methylation Test

*Dippmann C.[1], Schmitz M.[1], Hansel A.[1], Dürst M.[2]
[1]oncgnostics GmbH, Jena Germany;
[2]Department of Gynecology, Jena University Hospital, Jena, Germany

Results

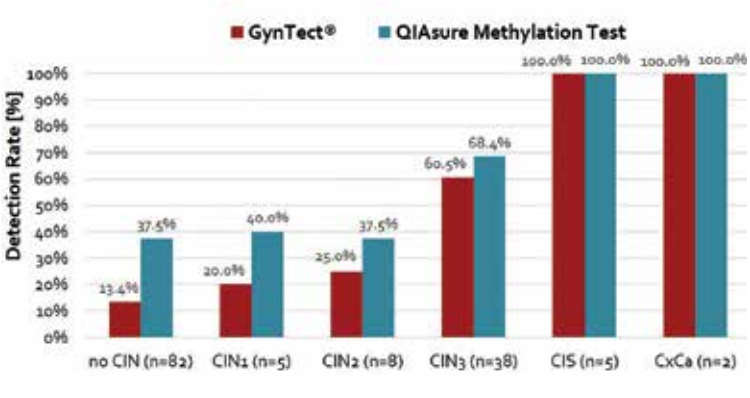


Figure B: Detection Rates of GynTect and QIAsure Methylation test on cervical scrapes.

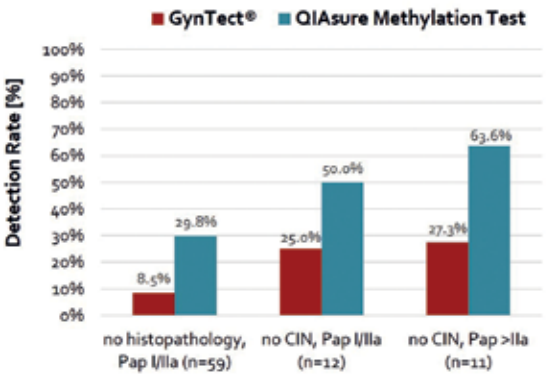


Figure C: Detection Rate of GynTect and QIAsure Methylation test, with the 'no CIN group' differentiated.

	GynTect®	QIAsure Methylation Test
Sensitivity		
CIN2+	60.4 %	67.9 %
CIN3+	66.7 %	73.3 %
Cancer	100 %	100 %
Specificity		
CIN2+	88.2 %	66.2 %
CIN3+	86.9 %	65.9 %

CIN3+	GynTect®	QIAsure Methylation Test
PPV	73.2 %	54.1 %
NPV	83.0 %	81.8 %

Conclusion

The detection rate for cervical cancer is 100 % for both assays. QIAsure Methylation Test shows a slightly higher sensitivity compared to GynTect®, whereas GynTect® has a circa 20 % higher specificity. Based on these data the PPV of GynTect® is higher, whereas both tests show a comparable NPV.

Table A: Sensitivity, specifi city, PPV and NPV of the two test systems regarding the detection of CIN2+, CIN3+ and cancer on a cohort of 129 HPV-positive samples. Excluded samples: no CIN,Pap >IIa (n=11)

Clarification testing/triage of women tested HPV DNA-positive in cervical cancer screening using a DNA methylation marker-based test as well as an HPV mRNA test*

Presenter. Anna-Bawany Hums
Molecular Biologist | oncgnostics GmbH

Objectives

HPV-testing is more and more implemented in cervical cancer screening resulting in a higher sensitivity. This is at the expense of specificity, which may result in overtreatment and higher screening costs.

Aim: Assess DNA methylation marker-based testing and HPV mRNA testing as reliable triage methods for clarification of HPV DNA-positive women. Additional testing may provide a suitable tool especially with respect to keeping false-positive rates low.

*Anna-Bawany Hums, Gabriel Campana, Kristin Knoll, Cordula Schuster, Alfred Hansel, Martina Schmitz, Joachim Zimmermann-Marroquin

Methods

Cervical smear samples (n = 231) with cytology findings ≥Pap III, in liquid-based cytology medium (BestPrep®; CellSolutions). For all samples results from HPV DNA testing (Roche Cobas HPV test) were available.

GynTect® (oncgnostics): DNA methylation assay for the detection of six tumour-specific DNA methylation marker

Aptima HPV mRNA assay (Hologic): in vitro detection of mRNA of the oncogenes E6/E7 from 14 hrHPV types allows detection of active infection

The number of positively tested samples increases between PAP III and PAP IV for both tests. In the group of Pap III samples the rate of positivity for the HPV mRNA test (Aptima) is much higher than for the DNA methylation assay GynTect® (Figure 1).

	Pap III (170)			Pap IV (53)			Pap V (5)		
HPV group (number)	samples	GynTect-positive	HPV mRNA-positive	samples	GynTect-positive	HPV mRNA-positive	samples	GynTect-positive	HPV mRNA-positive
HPV 16/18 (94)	56	42.9% (24/56)	67.9% (38/56)	35	85.7% (30/35)	85.7% (30/35)	3	100.0% (3/3)	100.0% (3/3)
HPV others (108)	90	13.3% (12/90)	76.7% (69/90)	17	70.6% (12/17)	88.2% (15/17)	1	100.0% (1/1)	100.0% (1/1)
HPV-negative (26)	24	12.5% (3/24)	4.2% (1/24)	1	0.0% (0/1)	0.0% (0/1)	1	invalid	negative

Table 1: Positivity of GynTect and Aptima HPV in relation to cytology findings

The difference between Aptima and GynTect® is most pronounced in women with Pap III cytology, infected with other types than HPV16/18 (Tab.1). Lesions based on infections with HPV types other than HPV16/18 have lower progression potential. Also samples from patients with high-grade histopathology-confirmed lesions based on infections with HPV types other than HPV16/18 are much less frequently GynTect-positive.

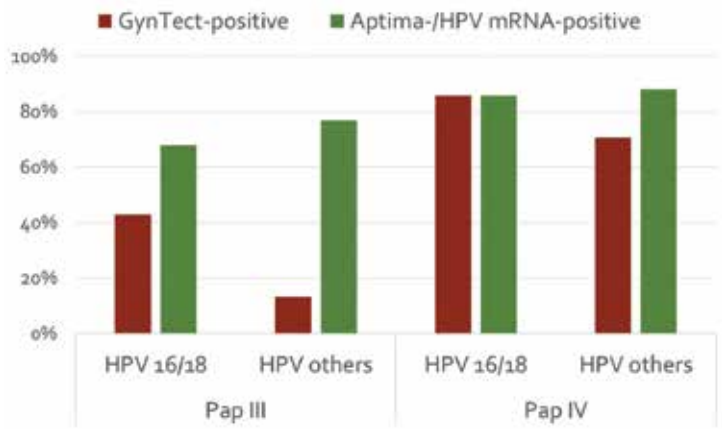


Figure 1: Positivity in relation to cytology and HPV DNA

Conclusion

Detection of mRNA of the HPV oncogenes E6 and E7 provides a sign for a persisting infection with HPV. In contrast, the DNA methylation markers comprising GynTect® are a direct sign for carcinogenesis. Triage using GynTect may show much higher specificity than using HPV mRNA. The main difference in positivity of both tests is seen in the group of samples that are HPV DNA-positive and show a cytology Pap III. Thus, GynTect may help to clarify the malignan- cy status of HPV-positive women with cytological signs of max. mild dysplasia.

Comparison of the performance of the DNA methylation marker test GynTect® and the CINtec Plus cytology assay*

Presenter: Dr. med. Ilona Zeiser
Gynecologist, Senior Cytologist,
Study Represeentative | CytoMol

Objectives

Reliable triage methods are essential in cervical cancer screening settings in order to avoid over- treatment and higher screening costs. Among the screening triage options discussed, specific DNA methylation patterns may provide a suitable tool especially with respect to keeping false-positive rates low.

Methods

GynTect® was performed on 1000 surplus cervical scrapes collected in PreservCyt® from women with cytology findings ≥ Pap III (ASC-H, AGC, LSIL, HSIL). For all samples data for cobas HPV test- ing with partial genotyping as well as p16/Ki-67 dual staining (CINtec Plus®) were available. For a subset of >600 samples, corresponding histopathology findings were available.

Results

- → Less than 20% of samples with cytology LSIL (Pap IIID1) were tested Gyn-Tect-positive
- → A larger proportion of samples with cytology HSIL (Pap IIID2, Pap IV; 38%) were tested Gyn-Tect-positive
- → GynTect results correlate with HPV genotypes:
  - Samples from women with HPV16/18 infection are more frequently GynTect-positive
  - Highest GynTect positivity is observed in women with high-grade cytology Pap IV (HSIL) and HPV16/18 infection (80%)
- → CINtec Plus was positive in a high proportion of LSIL (Pap IIID1) samples (>80%) as well as in most HSIL (Pap IIID2, Pap IV) samples

- → GynTect-positivity rises with severity of histopathology findings for the corresponding patients: almost 60% of CIN III and all cancers are methylation-positive
- → CINtec Plus does not allow differentiation between histopathology findings: overall >90% of samples with histopathology available are positive for p16/Ki67

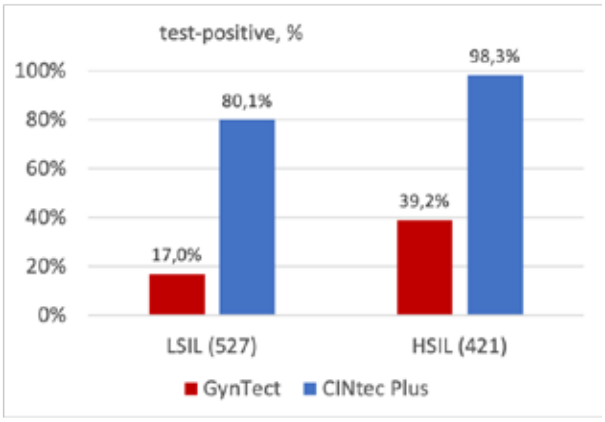


Figure A: detection rate of the two assays GynTect and CINtec Plus among samples from women with cytology findings Pap III or Pap IV.

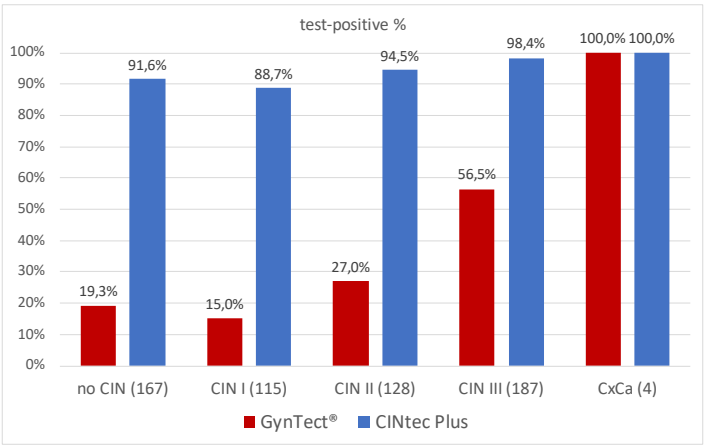


Figure B: Detection rates of the two tests related to histopathology findings, when available

Conclusion

The results imply that the six DNA methylation markers comprising the cervical cancer diagnostic GynTect, may be useful in clarification of women with abnormal and unclear Pap smear findings. As a molecular laboratory test GynTect functions independently of a human assessment, which may be subjective, e.g., in borderline cases.

GynTect® DNA methylation marker - longitudinal observational study in patients with CIN2/3. Results from the GynTect-PRO trial*

Presenter: Dr. Martina Schmitz
Biochemist | CSO oncgnostics GmbH

Objectives

Precancerous lesions of the cervix, depending on their grade of severity, are known to have a high potential to regress to normal. Especially among young women, only a few patients are at high risk to develop invasive cancer. DNA methylation markers such as ASTN1, DLX1, ITGA4, RXFP3, SOX17, ZNF671

* Martina Schmitz, Juliane Hippe, Kristina Wunsch, Alfred Hansel, Tabita Heller, Heike Hoyer, Matthias Dürst, on behalf of the study group

comprising the diagnostic test GynTect® are a new class of biomarkers which are discussed to have prognostic potential. GynTect-PRO is a prospective, longitudinal and multi-centered trial which addressed the probability of regression in GynTect-negatively tested women having a CIN2 or CIN3 lesion (Negative Predictive Value, NPV). The study tests the null hypothesis NPV≤70% against the alternative hypothesis NPV≥90%.

Methods

77 women <25 years were included between December 2017 and February 2021, recruited from nine clinical centers in Germany. All patients had a histopathology confirmed CIN2 or CIN3 lesion at first visit. Follow-ups were planned for all patients every 6 months, CIN2 patients for up to 24 months (a max. of 5 visits) and CIN3 patients for up to 12 months (a max. of 3 visits). At all visits, colposcopy was done and, if indicated, a biopsy was taken. HPV testing, cytology and GynTect testing was also done at every timepoint. The outcome was defined as progression, persistence or regression with respect to the last biopsy, conization or colposcopy findings if no histopathology was done at the last visit.

Results

60 women fulfilled all inclusion criteria.

CIN2 patient group: 24 patients were included (see Table 1); 18 were GynTect®-negative at V0.

- → 12 (66.7%) patients showed a regression
  NPV = 0.67, 90% CI 0.44 – 0.85, p=0.53
- → 6 (33.3%) showed a persistent lesion
- → N0 patient showed a progression

CIN3 patient group: 36 patients were included (see Table 2); 27 were GynTect®-negative at V0.

- → 15 (55.6%) patients showed a regression
  NPV =0.56, 90% CI 0.38 – 0.72, p=0.92
- → 12 (44.4%) showed a persistent lesion
- → N0 patient with status progression

CIN2 GynTect V_0	Outcome						Total	
	Regression		Persistence		Progression			
	n	%	n	%	n	%	n	%
Invalid	2	100	0	0	0	0	2	100
negative	12	66.7	6	33.3	0	0	18	100
positive	3	75.0	1	25.0	0	0	4	100
All	17	70.8	7	29.2	0	0	24	100

Table 1: CIN2: Median outcome CIN2 patients after 1.8 years (range 0.5 – 2.6 years).

CIN3 GynTect V_0	Outcome						Total	
	Regression		Persistence		Progression			
	n	%	n	%	n	%	n	%
Invalid	3	100	0	0	0	0	3	100
negative	15	55.6	12	44.4	0	0	27	100
positive	2	33.3	4	66.7	0	0	6	100
All	20	55.6	16	44.4	0	0	36	100

Table 2: CIN3: median outcome CIN3 patients after 1 year (range 0.2 – 1.4 years).

Conclusion

Majority of CIN2 and CIN3 lesions in this study showed regression during follow-up, but the hypothesis (NPV ≥ 90%, CI=0.9: NPV ≥ 0.7, 80% power) could not be confirmed.

Some of the study participants terminated the study prematurely.
If in such a case a final persistence is detected, the probability of a final regression might be underestimated and that of a final persistence/progression might be overestimated.
In a sensitivity analysis in which regression is assumed for prematurely terminated cases with persistence (<1.5 years for CIN2, <0.8 years for CIN3) the negative predictive value would be:

- → for CIN2 patients 0.78 (14/18) (90% CI 0.56 - 0.92, p right hand=0.33)
- → for CIN3 patients 0.70 (19/27) (90% CI 0.52 - 0.85, p right hand=0.58).

NMPA approval trial of Gong An Li (GynTect®), a DNA methylation assay using six biomarkers for detecting cervical cancer and its precancerous lesions*

Presenter: Dr. Martina Schmitz
Biochemist | CSO oncgnostics GmbH

Objectives

GynTect is an assay using six human DNA methylation biomarkers for detecting cervical cancer and its precancerous lesions. GynTect is CE IVD marked since 2015 to clarify HPV positive tested women and/ or women having abnormal cytology findings.
Here we present the outcome of the National Medical Products Administration (NMPA) approval trial for GynTect which was run in China between 2018 and 2021.

Methods

The trial was designed as a multicentered, cross-sectional trial in four diffenent hospitals in China: Beijing, Nanjing, Hunan and Anhui*.
Patients aged 21-65y were included and screened first line with HPV. Positively tested women were included in the study, and GynTect, liquid-based cytology (ThinPrep cytology testing = TCT), colposcopy and biopsy was done on all patients included. Each centre performed GynTect, HPV, TCT and colposcopy in the own facilities.

Results

Detection rates and clinical performance of GynTect and TCT were calculated on all HPV positive women, see Figure 1,2 and 3.
A slightly higher detection rate was seen for both, GynTect and TCT, for the non HPV16/18 group compared to only HPV16/18 positives (graph not shown).
Only in the histology “normal” group, GynTect showed higher (12% vs 5.8%) detection in the HPV16/18 group.

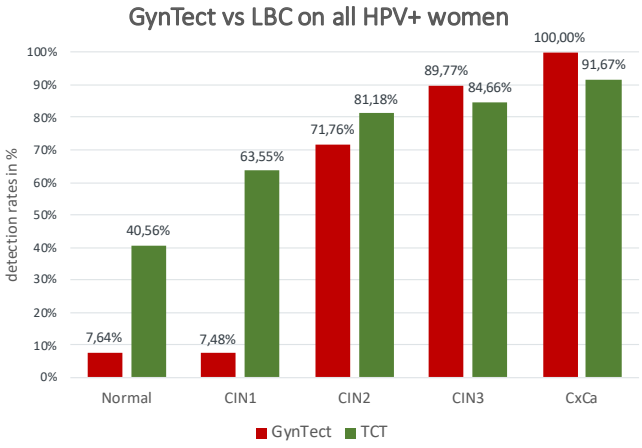


Figure 1

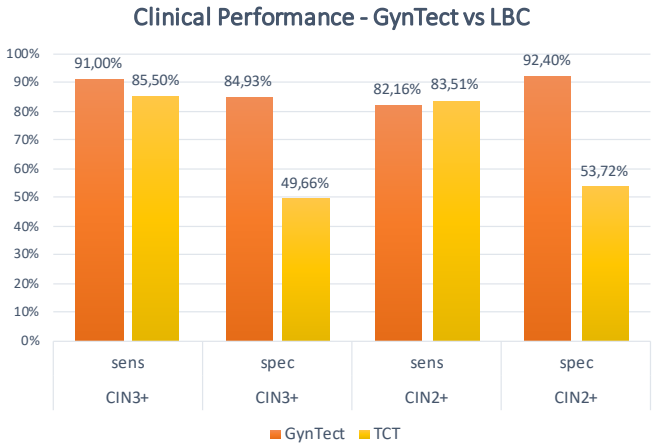


Figure 2

*Zhao Jian, Peking University First Hospital, China, Hong Yin, Nanjing Drum Tower Hospital, China, Zhao Jing, Maternal and Child Health Care Hospital Hunan, China Pan Xiaohua, The First Affiliated Hospital of Anhui Medical, China

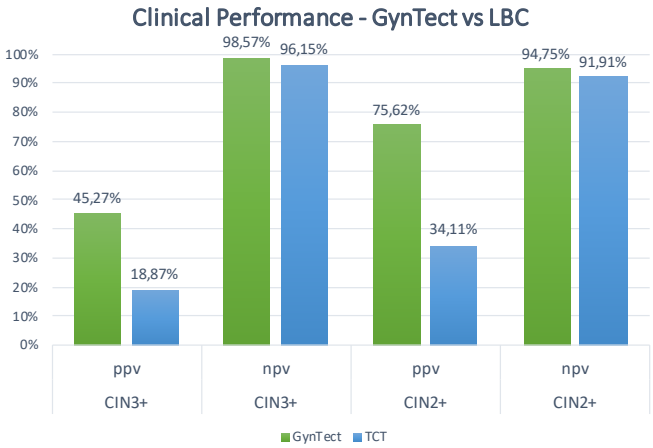


Figure 3

Conclusion

GynTect performance was superior compared to TCT for clarification of HPV positively tested women. → GynTect, which is named Gong An Li in China, is approved since 4th August 2022 for clarification of non-HPV16/18 positively tested women!

A German online survey of patients with CIN, highlighting the psychological distress during repetitive diagnostics cycles*

Presenter: Dr. Martina Schmitz
Biochemist | CSO oncgnostics GmbH

Objectives

HPV and cytology testing are well established screening methods, however they both are not able to distinguish between those lesions which may progress to cancer and those which may heal spontaneously.
Resulting from this, sequences of follow-ups create a burden to women as they will have to stand the ongoing uncertainty whether cancer is already in progress or not.
We designed a survey to address the question of psychological burden due to abnormal Pap smear results and/or positive HPV tests.

Methods

The online-survey had a semi-structured design, combining explorative questions with validated elements. Participants went through a 37-item survey including the IES-R („Impact of Event Scale-Revised" – German Version) as well as parts of the CDDQ (Cervical Dysplasia Distress) questionnaires. Participants

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[1]Hannover Medical School, Department of Gynaecology and Obstetrics, Hannover, Germany; [2]DontBePatient Intelligence GmbH, München, Germany; [3]Varigon GmbH, Hamburg, Germany; [4]oncgnostics GmbH, Jena, Germany

for the survey were recruited using online marketing (via Facebook and Google) and the community of "Myriam von M" on Facebook.

Results

Participants (n=3753) had a mean age of 31.8 years, and 35.3% were still in family planning. Almost half (46.6%) of the women indicated that they had 3 to 5 (32.1%) or even more (14.5%) abnormal Pap smears, but only 40.9% were aware of their actual Pap finding (table 1). 53.1 % of the women had already been affected for more than one year. 69.9% and 76.4% of the participating women reported that their worries about the Pap and HPV findings, respectively, were at least "quite a bit" (Scores 3,4 & 5 on a 5-point scale, figure 1). 48.1% of them stated that the risk of conizations as well as the risk of preterm birth is important to them and "clearly" to "severely" impacting their life (Scores 4 & 5, figure 2).

Question	Proportion of answers (%)
Yes, I know my actual Pap finding	40.9%
PAP I	21.0%
PAP II	20.2%
PAP III	13.3%
PAP IIID	27.6%
PAP IVA	8.7%
PAP IVB	2.0%
PAP V	0.9%
I'm not quite sure	6.6%

Table 1

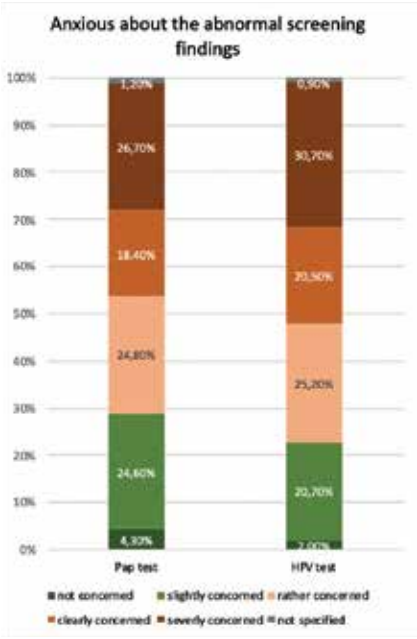


Figure 1

Figure 2

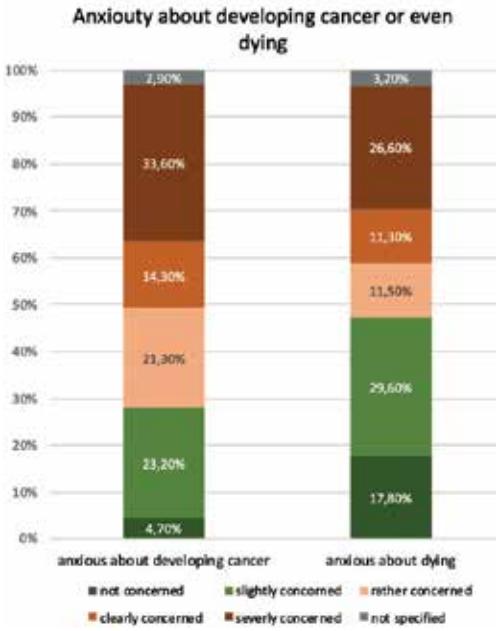


Figure 3

Even more alarming, 69.1% stated to be afraid of developing or being diagnosed with cervical cancer and 49.4% expressed that they were even anxious about dying (figure 3).

Conclusion

This survey is the first of its kind to investigate the psychological distress during repetitive diagnostics cycles from patients with abnormal Pap / HPV findings and highlights important findings in relation to the unmet needs for a clear prognosis or diagnosis in cervical cancer screening.

ScreenYu Gyn® - cervical cancer screening triage based on a single DNA methylation marker *

Presenter: Dr. Alfred Hansel
Biologist | CEO oncgnostics GmbH

Objectives

Different strategies are discussed regarding triage/confirmation tests in HPV-based cervical cancer screening, among them DNA methylation markers. Detecting DNA methylation is, however, not yet automated, and detecting a set of markers is still expensive and time-consuming. The duplex QPCR assay ScreenYu Gyn based on the single DNA methylation marker ZNF671 plus a control marker to detect cervical cancer and its relevant precancerous lesions using cervical scrapes, is a first step towards a simplified test.

Methods

With ScreenYu Gyn the cancer marker ZNF671 and, as a quality control, ACTB are detected in a single-tube reaction after bisulfite conversion (EZ DNA Methylation Lightning Kit, Zymo Research) of the clinical sample. One mL of a cervical smear collected in PreservCyt medium (ThinPrep, HOLOGIC) is used for bisulfite treatment, and 10µL of the 15µL eluate are used for the PCR. The test is CE IVD-marked for the cobas Z480 (Roche Diagnostics) and the CFX96 (BioRad Laboratories) QPCR plattforms.

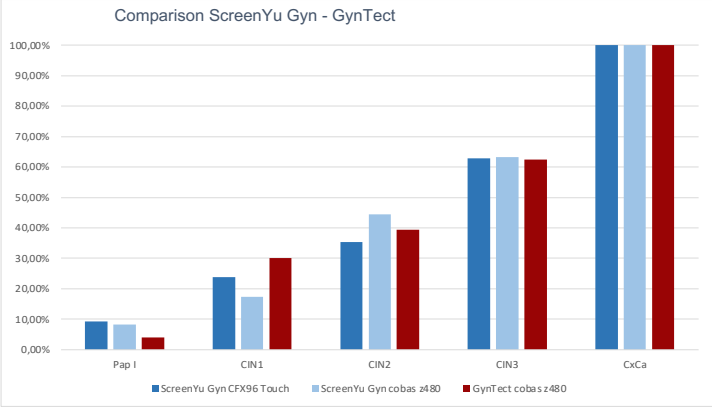


Figure: detection rate of the two assays ScreenYu Gyn and GynTect, determined on independent sample collections, respectively. For both studies residual samples from routine cervical screening, collected in LBC medium, were used (ScreenYu Gyn 616 samples; GynTect 320 samples).

Results

- → Oncgnostics' newly developed cervical cancer diagnostic assay ScreenYu Gyn has high analytical sensitivity and specificity
- → The clinical performance of ScreenYu Gyn is very good:
  - → Sensitivity for CIN3+ is 65%, similar to GynTect
  - → Specificity among NILM samples >90%

Conclusion

ScreenYu Gyn provides the basis for an easier to perform DNA methylation marker assay for use in cervical cancer diagnostics. The CE IVD-marked assay allows for higher throughput, but still includes bisulfite treatment. Further research and development activities aim at a point of care solution for application also in underserved regions, where the burden of cervical cancer still is highest.

*Martina Schmitz, Juliane Hippe, Kristina Wunsch, Katja Schubert, Dana Schmidt, Anne Hennig, Alfred Hansel; oncgnostics GmbH, Jena, Germany

Assessment of DNA methylation markers for the detection of VIN, VAIN and vulva or vagina carcinoma*

Presenter: Dr. Alfred Hansel
Biologist | CEO oncgnostics GmbH

Objectives

The majority of vaginal cancers and a lesser proportion of vulvar cancers are associated with HPV. Rising incidence rates of vulvar cancer seem to be mainly attributable to high-risk HPV-positive cases. Currently, no organized screening is performed in any country, so early detection is often an incidental finding during routine visit at the gynecologist.

Methods

Scrapes from patients with vulvar and vaginal carcinoma as well as vulvar (VIN) and vaginal intraepithelial neoplasias (VAIN) were analyzed for methylation of the six marker regions ASTN1, DLX1, ITGA4, RXFP3, SOX17 and ZNF671 comprising the cervical cancer diagnostic test GynTect.

Results

Among 37 Vulva cancers 16 were HPV-positive, 21 HPV-negative

→ 15 of 16 HPV-positive vulva cancers were DNA methylation-positive

→ 13 of the 21 HPV-negative vulva cancers were DNA methylation-positive

All 6 Vagina cancers were HPV-positive

→ All 6 samples were GynTect-positive

Absolute GynTect results on all 28 VIN/VAIN samples with valid tests
19 samples were GynTect-positive

→ All GynTect-positive samples were HPV-positive

→ The four HPV-negative VINs were also GynTect-negative

→ Of the 16 VINs with valid GynTect result, 15 were GynTect-positive; two of the four „invalid" samples were positive each for 5 markers

→ Of the 8 VAINs – all HPV-positive - 4 were GynTect-positive

Histopathology	samples	HPV-positive	HPV-negative
VIN	25	21	4
Vulva Ca	37	16	21
VAIN	8	8	0
Vaginal Ca	6	6	0

Table 1

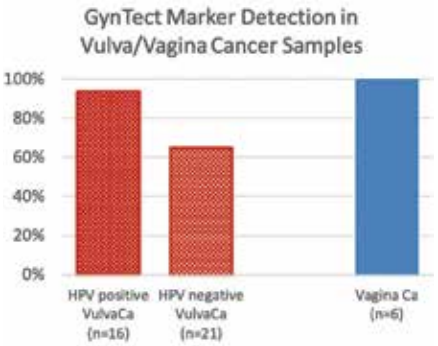


Figure 1

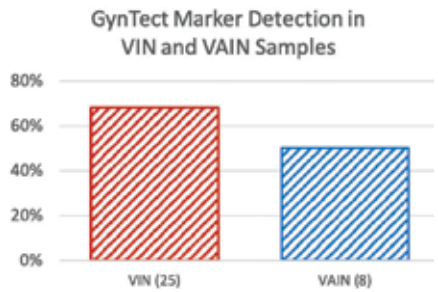


Figure 2

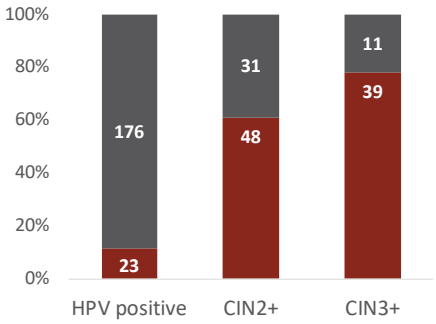
Conclusion

The six DNA methylation markers comprising the cervical cancer diagnostic GynTect, may be useful in diagnostics of vulvovaginal diseases as well, as a high proportion of the samples were positive for GynTect, partially irrespective of the HPV status. Thus, in combination with HPV diagnostics, DNA methylation testing with these markers might be a promising tool for early detection of malignant vulvar or vaginal disease.

*Alfred Hansel[1], Kristina Wunsch[1], Martina Schmitz[1], Annett Petzold[2], Matthias Dürst[2];
[1]oncgnostics GmbH, Jena, Germany,
[2]Department of Gynaecology and Obstetrics, University Hospital Jena, Jena, Germany

Evaluation of host gene methylation as a triage test for HPV positive women in a real-world clinical setting

Presenter: Mariana Costa
Biologist | UniLabs LAP Porto



Background

High-risk HPV tests are the mainstay for the screening of cervical cancer and its precursors, replacing cytology as the primary screening test in appropriate settings and age cohorts. These tests have, however, a moderate specificity and positive predictive value, as most positive cases represent prevalent HPV infection. DNA methylation has high sensitivity and specificity for cervical intraepithelial neoplasia (CIN) 2+, and especially for invasive cervical cancer and may thus provide a possible triage in HPV-based cervical cancer screening settings.

Objectives

We evaluate the impact of a strategy of triage of all HR-HPV positive cases with a methylation assay (GynTect®) in an organized cervical cancer screening program, in comparison to routine standard of care, as well as the possible influence of the variation of the rate of HPV16/18 in its performance.

Triage strategy	% of HPV positive women referred for colposcopy	Number of colposcopies for diagnosis		Detection rate	
		CIN2+	CIN3+	CIN2+	CIN3+
HPV16/18	32.7%	2.2	2.9	51.2% (41/79)	62% (31/50)
Methylation	25.5%	1.5	1.8	60.8% (48/79)	78.0% (39/50)
HPV16/18 and other HR-HPV methylation positive	43.2%	2.0	2.7	74.7% (59/79)	90% (45/50)

Methods

Cohort study with consecutive women referred for colposcopy in an organized cervical cancer screening program who had colposcopy, biopsies and repeated HPV testing. HPV-positives at the time of colposcopy were tested with GynTect. The performance of the test was evaluated and compared to standard practice.

Results

The DNA methylation marker test had a sensitivity and specificity for CIN2+ of 60.8% (49.1-71.6%) and 88.4% (83.2-92.5%), respectively. For CIN3+, it was of 78.0% (64.0-88.5%) and 86.0% (80.8-90.2%), respectively (Figure: GynTect detection rates/histopathology CIN2+/CIN3+).
The rate and level of methylation positively correlated with the severity of disease. The use of methylation reduces the referral for colposcopy to 25.5%, while detecting 78.0% of the CIN3+ cases. Referral of all HPV16/18 positive cases and triaging the other high risk HPV positive cases with methylation, detects 90.0% of the cases of CIN3+, while reducing the number of referrals to 43.2% (see Table).

Conclusions

The GynTect® methylation test has a high sensitivity and specificity for CIN3+ and significantly reduces the rate of referrals for colposcopy. Sending of all women screened HPV16/18-positive directly to colposcopy and among the other HR-HPV positives the ones tested methylation-positive, may be the best option to reduce the number of referrals for colposcopy, while detecting 90% of the cases of CIN3+.

*Pedro Vieira-Baptista, Mariana Costa, Juliane Hippe, Carlos Sousa, Martina Schmitz, Ana-Rita Silva, Alfred Hansel, Mario Preti

GynTect® Methylation Markers detect recurrent disease in patients treated for CIN3 with high sensitivity and specificity in a retrospective case-control study

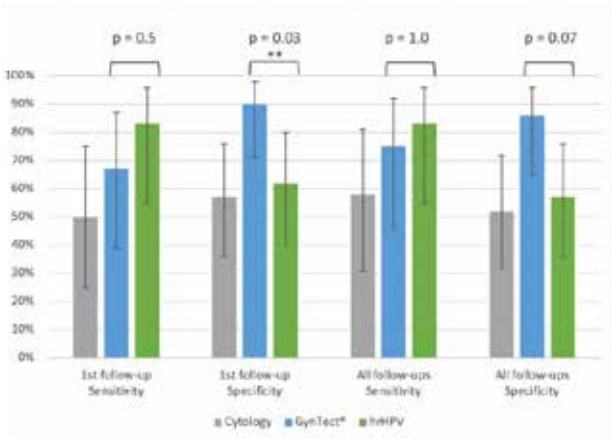
Presenter: Dr. Alfred Hansel
Biologist | CEO oncgnostics GmbH

Background

Post-treatment follow-up in women with high-grade cervical lesions (CIN3) is mandatory due to relapse in up to 10% of patients. Standard follow-up based on hrHPV-DNA/cytology co-testing has high sensitivity but limited specificity. The aim of our case-control study was to evaluate the performance of the methylation markers comprising the cervical cancer diagnostic GynTect® as a means for the timely detection of recurrent CIN2/3 during follow-up.

Methods

Residual clinical material from an observational study with a focus on HPV/cytology co-testing was analysed (PMID34282754). We studied a sample of 48 patients (median age 31 years) comprising 17 cases with recurrent CIN2/3 diagnosed within 24 months and 31 controls. All of them had at least one follow-up visit. DNA from cervical scrapes at baseline (before CIN3 surgery) and follow-up visits were analysed for 13hrHPV types and the GynTect® methylation status.



Results

Overall, 15 of 48 patients were GynTect-negative at baseline. Of the 33 GynTect-positive patients at baseline 12 were diagnosed with recurrent disease. Two of these patients were neither hrHPV- nor GynTect-positive during follow-up. One patient was hrHPV-positive but GynTect-negative and 9 patients were hrHPV- and GynTect-positive during follow-up.

Sensitivity was not significantly different for GynTect (75%, 95% CI 46%-92%) and hrHPV (83%, 95% CI 55%-96%) (McNemar p=1.00). Two of 21 patients who were GynTect-positive at baseline but without evidence for recurrent disease were both hrHPV- and GynTect-positive during follow-up. Six patients were hrHPV-positive but GynTect negative and 13 patients were negative for both tests. Specificity was significantly higher for GynTect (90%, 95% CI 71%-98%) compared to hrHPV (62%, 95% CI 40%-80%) (McNemar p=0.03).

Conclusions

For initially GynTect-positive patients both hrHPV and GynTect® assays detect recurrent disease with similar sensitivity but the GynTect® assay has a significantly higher specificity. A future study will have to show whether cytology/GynTect® co-testing will out-perform cytology/hrHPV co-testing in post-treatment surveillance for this subgroup of CIN3 patients.

Heike Hoyer, Cornelia Scheungraber, Grit Mehlhorn, Ingke Hagemann, Sarah Scherbring, Linn Woelber, Annett Petzold, Kristina Wunsch, Martina Schmitz, Monika Hampl, Gerd Böhmer, Peter Hillemanns, Ingo B Runnebaum and Matthias Dürst

Direct comparison of the performances of the single-marker assay ScreenYu Gyn and the six-marker assay GynTect

Presenter: Dr. Martina Schmitz
Biochemist | CSO oncgnostics GmbH

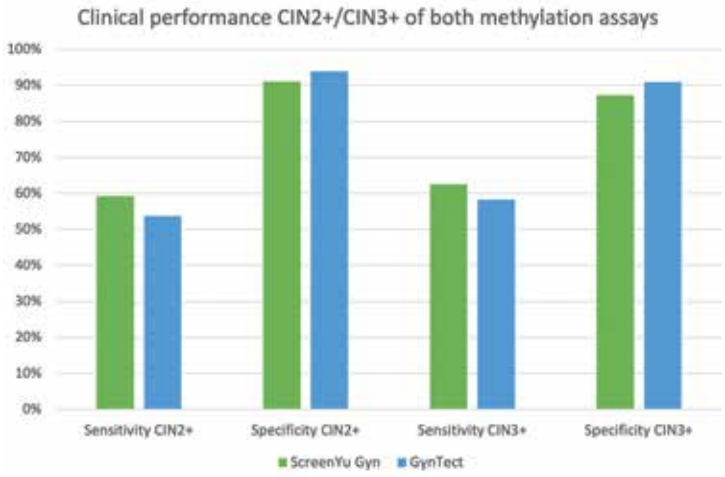
Background/Objectives
Accurate clarification of women with HPV positive test results or abnormal cytology findings during routine screening is important in order to reduce both, overtreatment as well as long, psychologically stressful watchful waiting periods. Different strategies are discussed, among others DNA methylation markers show a high potential. Detecting DNA methylation is, however, not yet automated, and detecting a set of markers is still expensive and time-consuming. We compared GynTect, comprising six methylation markers (ASTN1, DLX1, ITGA4, SOX17, RXFP3 and ZNF671) with ScreenYu Gyn, based on a single methylation marker (ZNF671), regarding detection of cervical precancerous lesions and cancer using cervical scrapes.

Methods
GynTect is a Eva-Green based singleplex real-time PCR for the detection of six methylation markers ASTN1, DLX1, ITGA4, SOX17, RXFP3 and ZNF671, as well as two control marker regions ACHE and IDS-M, using bisulfite converted (EpiTect Fast DNA Methylation Kit, Qiagen) clinical samples. ScreenYu Gyn is a duplex methylation-specific real-time PCR assay using TaqMan probes for the detection of the amplicons. ZNF671 as methylation marker and ACTB as quality control are amplified in a single-tube reaction after bisulfite conversion (EZ DNA Methylation Lightning Kit, ZymoResearch) of the clinical sample. To compare clinical performance between these two assays, a clarification cohort comprising >600 residual cervical scrapes, collected in Thinprep PreservCyt (HOLOGIC), were analyzed with both assays.

Results

Valid results were obtained in both assays for 611 samples: 334 NILM, 46 CIN1, 44 CIN2, 179 CIN3 and eight cervical cancer samples. Both assays detected all eight cervical cancer samples. Among high grade cervical lesions, ScreenYu Gyn showed a slightly higher detection rate than GynTect: 60.9% and 45.5%for CIN3 and CIN2, vs. 56.4% and 34.1%, respectively. Detection rate for CIN1 samples was 17.4% with ScreenYu Gyn and 19.6% with GynTect. The detection rate in the NILM group was slightly higher for ScreenYu Gyn with 7.8% compared to GynTect with 4.2%.
However, none of the different detection rates regarding the histological group was significantly different according to Chi2 assessment.

Samples	Detection rate ScreenYu Gyn	Detection rate GynTect	p-value (Chi² calc.)
NILM (n = 334)	7.8%	4.2%	p-value = 0.07285
CIN1 (n = 46)	17.4%	19.6%	p-value = 1
CIN2 (n = 44)	45.5%	34.1%	p-value = 0.3836
CIN3 (n = 179)	60.9%	56.4%	p-value = 0.4525
Cancer (n = 8)	100%	100%	n.a.



Conclusions

GynTect shows a slightly better specificity, whilst ScreenYu Gyn has a slightly better sensitivity for CIN2+ and CIN3+ detection. Overall, both assays showed concordant results in 91.2% of the assays, according to Cohen's kappa assessment an almost perfect agreement. The single-marker assay ScreenYu Gyn has clear advantages regarding hands-on time and overall duration. It provides an excellent basis for further development of an even simpler and automatable DNA methylation marker test for cervical cancer diagnostics.

WM_028-01

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