Evaluation of the diagnostic accuracy of a candidate biomarker to detect use of conventional cigarettes in users of tobacco products that do not burn tobacco

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Introduction

For studies assessing the effects of modified tobacco products, a compliance marker to assess a subject's product compliance is needed. This biomarker is intended to enable the identification of subjects participating in clinical or epidemiological studies that use, or have recently used, conventional cigarettes (CCs) in addition to modified tobacco products (MTPs).

In a previous in-house study (PMRL-study 11621), *N*-acetyl-*S*-(2-cyanoethyl)cysteine, otherwise known as 2-cyanoethylmercapturic acid (CEMA), a urinary mercapturic acid metabolite of acrylonitrile, was identified as a promising candidate compliance marker which may have the capacity to identify smokers who smoked CCs. Subsequently, a quantitative analytical method for CEMA was developed and validated.

In order to gather preliminary data using the validated analytic method and to evaluate the diagnostic perfomance in detecting CC smokers, banked urine samples from a clinical study (YVD-CS01-EU, NCT NCT00812279) were analyzed.

Materials and methods

The clinical study YVD-CS01-EU was a randomized, controlled, open-label, 3-arm parallel single center confinement study to investigate exposure to selected smoke constituents in smokers switching from CCs to MTPs or to smoking cessation (SC) for 5 days.

This study was run in Poland from 20 Nov. 2008 to 4 Feb. 2009. Healthy Caucasian adult smokers (male and female), age 23 to 55, who usually smoked 10 to 30 CCs (with a maximum ISO tar yield of 10 mg) per day, for at least the last 5 consecutive years, were randomized to 3 study arms as shown in Table 1.

Table 1: Study Arms and Planned Number of Subjects

Study arm	Description	No. of subjects randomised
MTP	Modified Tobacco Product (no limit on consumption)	56
CC	CC with ISO tar yield up to 10 mg (no limit on consumption)	28
SC	Smoking cessation	28

24-hour urine samples were collected from all subjects enrolled in the study for the measurement of 9 urinary biomarkers of exposure on 2 baseline days (Day -1, Day 0) and 5 investigational period days (Days 1-5).

In total, 749 samples from 107 subjects were analyzed for CEMA. The validated analytical method used to measure CEMA was a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with a lower limit of quantification (LLOQ) of 2 ng/ml and an upper limit of quantification (ULOQ) of approximately 500 ng/ml.

Data analysis and evaluation of the diagnostic performance was performed for 3 analysis variables, i.e., CEMA excreted in 24 hours (CEMAAe24h), CEMA concentration (CEMAc), and CEMA adjusted by urine creatinine (CEMAcreat), based on data from subjects in the per-protocol (PP) population and having CEMA values on all study days (105 subjects).

Results

CEMA levels in the 3 study arms were comparable at baseline with respect to all 3 CEMA variables (CEMAAe24h, CEMAc, and CEMAcreat). Baseline levels of all variables by study arm are shown in Table 2.

Table 2: Baseline Levels of CEMA Analysis Variables

		MTP (N=52)	CC (N=25)	SC (N=28)
Variable	**Unit**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
$CEMA_{Ae24h}$	µg/24h	221.45 (87.13)	216.50 (97.94)	203.80 (103.34)
$CEMA_c$	µg/l	131.79 (66.23)	134.00 (65.14)	126.70 (74.92)
$CEMA_{creat}$	µg/g creat	155.94 (51.12)	160.83 (55.94)	148.18 (56.96)

The averages of the 3 CEMA analysis variables over the course of the study in the study arms are summarized for the PP population in Table 3. A graphical presentation of CEMAcreat over the course of the study is provided in Figure 1.

Table 3: CEMA Analysis Variables over the Course of the Study

Study Arm	Day	$CEMA_{Ae24h}$ (µg/24h) Mean (SD)	$CEMA_c$ (µg/l) Mean (SD)	$CEMA_{creat}$ (µg/g creat) Mean (SD)
CC (N=25)	DAY -1	201.00 (88.62)	134.35 (69.09)	145.42 (51.51)
	DAY 0	232.00 (114.68)	133.36 (66.88)	176.24 (63.30)
	DAY 1	213.50 (91.04)	145.29 (60.26)	162.32 (60.70)
	DAY 2	230.46 (97.59)	152.68 (78.87)	173.27 (62.21)
	DAY 3	217.42 (87.28)	128.15 (60.92)	164.13 (59.12)
	DAY 4	226.59 (99.25)	144.56 (74.82)	163.79 (63.28)
	DAY 5	260.01 (204.78)	165.69 (89.66)	208.43 (123.60)
MTP (N=52)	DAY -1	217.86 (92.85)	133.87 (73.60)	145.27 (47.80)
	DAY 0	225.05 (95.37)	129.72 (65.00)	166.62 (61.60)
	DAY 1	81.10 (41.96)	50.01 (29.35)	57.46 (23.62)
	DAY 2	47.39 (22.51)	27.57 (18.16)	34.20 (13.70)
	DAY 3	38.60 (19.18)	19.60 (11.21)	27.44 (12.23)
	DAY 4	36.77 (18.40)	19.85 (11.68)	25.42 (11.30)
	DAY 5	32.87 (16.05)	18.98 (12.31)	25.38 (11.33)
SC (N=28)	DAY -1	209.72 (111.11)	132.99 (86.27)	143.32 (54.99)
	DAY 0	197.89 (101.96)	120.40 (68.87)	153.03 (61.05)
	DAY 1	81.36 (45.39)	52.31 (35.68)	60.22 (27.79)
	DAY 2	53.79 (39.52)	28.31 (25.74)	38.50 (22.92)
	DAY 3	44.41 (34.23)	21.66 (21.23)	31.96 (21.85)
	DAY 4	38.39 (27.45)	21.84 (19.34)	28.05 (17.48)
	DAY 5	36.97 (28.57)	21.36 (19.22)	28.98 (19.81)

Results cont'd

For all CEMA analysis variables, the reductions observed in the MTP study arm were similar to those observed in the SC study arm, whereas slight increases were observed in the CC study arm.

Figure 1: CEMAcreat over the Course of the Study

Already from Day 2, the levels of all three CEMA variables in the MTP and SC study arms were substantially lower than in the CC study arm.

These trends were similar for both cigarette consumption subgroups, while the levels in the ≥ 20 cigarettes per day (cpd) consumption groups remained slightly higher than in the 10-19 cpd groups. This was still seen in the SC study arm after 5 days, even though subjects did not smoke.

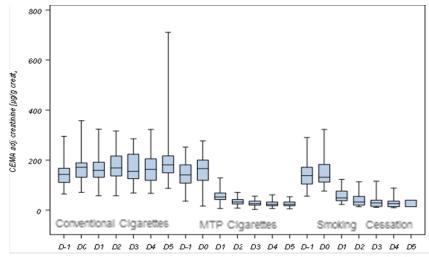
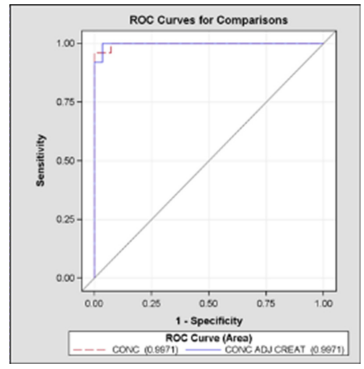


Figure 2: ROC Curves CEMAc and CEMAcreat (CC vs. SC, Day 5)



The Receiver Operator Characteristics (ROC) curve in Figure 2 (CC and SC data) shows the ability of CEMAcreat and CEMAc to distinguish between smokers who are currently smoking (CC study arm) and smokers who have not smoked for 5 days (SC study arm). The AUC of 0.99 is close to the maximum. Additional ROC analysis performed on data from the MTP and CC study arms also resulted in an AUC of 1.0 (separation of data points from both study arms).
In addition, 4 preliminary potential thresholds to characterize subjects as exposed or non-exposed to CC were calculated for CEMAcreat based on data from Day 5 from the CC and SC study arms. These thresholds ranged from 21.6 to 37.0 µg/g creat.

Tested using Day 2 and Day 5 data of subjects from the CC and SC study arms, all thresholds had a specificity of 1.0 (correctly identified subjects in the SC study arm) but a sensitivity of 0.58 to 0.69 (i.e., 30% to 40% of CC smokers not correctly identified) and an overall accuracy of 0.66 to 0.79.

Summary and conclusion

The distribution of this CEMA was close to normal allowing parametric methods to be used. The mean baseline level of CEMA in each of the 3 study arms was similar. The between-subject variability of CEMA was approximately 8 times higher than the within-subject variability.

In the CC study arms, levels increased over the course of the study. This has been seen in previous confinement studies for other biomarkers of exposure. In the SC and MTP study arms, levels at the end of the study were lower than at baseline. Statistical comparisons of the end-of-study values between the study arms indicated that levels were substantially lower in both the SC and MTP study arms than in the CC study arm. These trends were observed for all CEMA variables.

There was no overlap between the MTP study arm and the CC study arm at Day 5, with only limited overlap between the SC and CC study arms. CEMA was still quantifiable in the SC study arm after 5 days of non-smoking. This is consistent with a similar study in which levels of CEMAAe24h decreased to approximately 10% of their initial value after 8 days of non-smoking (Scherer et al., 2010). This indicates that there may be either alternative sources of acrylonitrile, another precursor of CEMA, or a sustained release of CEMA from the tissues.

In conclusion, CEMA performed well to discriminate CC smokers from subjects who switched to MTPs or to SC for 5 days. The CEMA analysis variable that appears to be the most suitable for the purpose of detecting subjects who smoke CCs in addition to MTPs is CEMAcreat, because it could be used for spot urine and 24 hour urine samples.

Further evaluation is necessary to evaluate the performance of CEMA at low cigarette consumption and to find suitable thresholds to distinguish subjects who recently smoked CCs from compliant smokers of MTPs.

References

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