Adenosine Concentration in Patients With Neuurally Mediated Syncope

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Background: Either high or low values of adenosine blood level (ABL) can differentiate some forms of neuurally mediated syncope (NMS). A rapid method of measurement has recently been developed. The aim of the present study was: (1) to compare ABLs in an unselected population of consecutive patients referred for evaluation of suspected NMS syncope and in healthy controls; and (2) to assess the relative prevalence of low and high adenosine forms among an unselected syncope population.

Method: Whole blood was collected after finger puncture, blood being deposited on a blot paper and adenosine concentration was measured by liquid chromatography/mass spectrometry (LC-MS/MS).

Results: Among 89 control subjects, the median ABL value was 0.54 µM (IQR, 0.46–0.65). The lowest 5% and the upper 95% percentile were 0.40 and 0.80 µM, respectively. Compared with healthy subjects, the 146 patients with syncope showed, on average, a higher median ABL value [0.63 (IQR 0.45–0.73, p = 0.04)] and a larger distribution of values. Low ABL values below the 5th percentile were observed in 28 (19%) patients, and, in five controls, p = 0.003 and high ABL values were observed in 26 (18%) patients and five controls, p = 0.009.

Conclusions: ABL is different in patients with suspected NMS than in healthy subjects. Patients with low and high adenosine values account for 19% and 18% of the general population. Thus, low and high ABL limits, as defined in this study, may help to define the purinergic profile of unselected subjects with a clinical diagnosis of suspected NMS.

Keywords: adenosine, syncope, neurally-mediated syncope, methodology, adenosine receptors

INTRODUCTION

Either central or peripheral baroreceptor reflex abnormalities and/or alterations in neurohumoral mechanisms could play a pivotal role in the genesis of extrinsic (functional) and neurally mediated syncope (NMS) (1). Among the several biochemical mediators that are advocated to play a role, adenosine has recently been investigated in typical vasovagal syncope and in syncope without prodromes in subjects with a normal heart. Either high- or low-adenosine values have been reported to be able to differentiate some forms of syncope, but adenosine does not seem to be involved in other forms. Schematically, high-adenosine and low-adenosine forms have been identified (2). When baseline adenosine is low, the effect of adenosine on the AV node and the sino-atrial node is mainly due to the stimulation of high-affinity A₁ receptors, (A₁R) which are...
Blood was collected using finger puncture, while the patient was
of blood (20 µL). The day of blood sampling, the participants were allowed to take
blood samples extraction with mixture, consisting of methanol and internal
standard solutions, and mixed overnight at room temperature to obtain
whole blood. Plasma was then transferred into an HPLC auto sampler vial.

**Blood Samples Extraction**

The method has been previously described (5). Briefly, six
dimensions of dried blood spot were cut out, followed by
evaluation of low-affinity A2A receptors (A2A-R) that are in the vessels and which
cause vasodilation (2).
The prevalence of patients with low and high adenosine
syncope is uncertain because, until now, adenosine has been
studied in small, selected populations. A barrier to the widespread evaluation of adenosine in clinical practice has
been the difficulty of obtaining rapid reliable measures. Indeed,
owing to its short half-life in the blood, adenosine is not
easy to sample and measure. At the time of venepuncture, a
stop solution – not commercially available - must be utilized
to inhibit the degradation of adenosine in body fluid. After
plasma deproteinization, adenosine concentration is evaluated
by means of high-performance liquid chromatography. A rapid
method (“blot spot”) has recently been developed and assessed
in a small population (5). It consists of measuring adenosine
concentration in whole blood instead of plasma, using fixed
potential amperometry. This method is rapid, well-accepted by
the patient, not expensive, and easily replicable.
The aim of the present study was: (1) to compare ABLs
in an unselected population of subjects with a history of
suspected NMS and in healthy controls; and (2) to assess the
relative prevalence of low and high adenosine forms among
this population.

**METHODS**

We assessed the rapid method of adenosine dosage in whole
blood using Liquid Chromatography, Mass Spectrometry (LC-
MS/MS) (5) in unselected consecutive patients with a history of
suspected NMS and in healthy volunteers. The blood samples
were shipped to the Laboratory of Biochemistry of Timone
Hospital for analysis.
The Syncope group was formed by patients aged >14 years
referred to the syncope clinic of Istituto Auxologico Italiano,
Milan, Italy because affected by one or more episodes of
unexplained syncope to confirm or reject the clinical suspicion
of NMS after exclusion of competing diagnoses. In particular,
according to the diagnostic criteria of ESC guidelines (6), the
patients with likely cardiac syncope and those with a transient
loss of consciousness of likely non-syncope origin were excluded.
The patients underwent head-up tilt test (HUTT) according to
the Italian protocol (7).

**Blood Samples Collection**

The day of blood sampling, the participants were allowed to take
their usual drug therapies and had no dietary restrictions. Whole
blood was collected using finger puncture, while the patient was
sitting in a quiet environment, followed by deposit of a drop
of blood (20 µL) on a blotting paper (Whatman 903 protein
saver cards™) and dried overnight at room temperature to obtain
dried blood spot. Then, the blot spots were stored at room
temperature and shipped to the Laboratory of Biochemistry of
Timone Hospital for analysis.

**Adenosine Dosage**

After the extraction, adenosine concentration was measured by
LC-MS/MS. Samples were analyzed using a Shimadzu UFLC
XR system (Shimadzu, Marne la Vallee, France). The LC system
was interfaced with an ABSciex 4,500 triple quadrupole mass
spectrometer (Les Ulis, France), operating with an electrospray
ionization source (ESI) using nitrogen (purity: 99.99%). Ten
microliters of the extracted sample were injected onto a 2.1-mm-
× 100-mm, 3-µm Atlantis T3 column, Waters (Guyancourt,
France). The starting mobile phase consisted of 3% methanol
and 97% acidified water (0.1% formic acid), with a flow of 0.7
ml/min for 3.5 min. Then, the gradient of methanol was increased
to 30% for 3 min. The column was re-equilibrated for 2 min to
starting conditions.

**Statistical Analysis**

ABL values were reported as median and interquartile range
(IQR) and compared between groups by means of Mann–
Whitney non-parametric test. Categorical variables were shown
as absolute and relative frequencies and compared between
groups by means of the Fisher exact test or Chi-squared test for a
trend as appropriate.

**RESULTS**

The study population consisted of 146 patients with a diagnosis
of likely neurally mediated syncope; their mean age was 57 ±
20 years, 67 were males (46%). The clinical features of patients
with syncope were consistent with those of an unselected general
population: 108 had prodromes, suggesting an autonomic
activation, 54 had recognizable triggers (orthostatic, emotional,
situational or post-prandial, single or in combination); 55 were
taking antihypertensive drugs. HUTT was positive in 101 (72%)
patients (vasodepressor or mixed in 88 and cardioinhibitory
in 13) and negative in 45 (28%) patients. The control group
consisted of 89 healthy volunteers; their mean age was 51 ± 18
years, 46 were males (52%).

In controls, the average median ABL value was 0.54 µM (IQR,
0.46–0.65). The lowest 5th and the highest 95th percentiles were
0.40 µM and 0.80 µM, respectively; these values were, therefore,
considered as the normal limit for low ABL and high ABL in
patients with syncope. Table 1 shows the results of ABL dosage
in both groups. Compared with healthy subjects, the patients
with NMS showed, on average, a higher median ABL value [0.63
(IQR, 0.45–0.73, p = 0.04)] and a larger distribution of values
(Figure 1). The low ABL zone included 28 (19%) patients and
TABLE 1 | Comparison of adenosine values between healthy controls and patients with syncope by the blot spot method.

|                         | Healthy subjects \( (n = 89) \) | Syncope patients \( (n = 146) \) | Odds ratio | \( P \)-value |
|-------------------------|----------------------------------|----------------------------------|------------|--------------|
| ABL, µM, median (IQR)   | 0.54 (0.46–0.65)                 | 0.63 (0.45–0.73)                 | 0.04       |
| No. of pts with ABL < 0.40 µM | 5 (5.6%)                        | 28 (19%)                         | 4.0 (1.5–10.7) | 0.003        |
| No. of pts with ABL > 0.80 µM | 5 (5.6%)                        | 26 (18%)                         | 3.6 (1.3–9.9)  | 0.009        |

FIGURE 1 | Distribution of patients with syncope and healthy controls based on their adenosine levels. The blue horizontal line shows the median; the red horizontal lines show the 5th and the 95th percentiles of the two groups. The low adenosine zone and the high adenosine zone, based on the normal ABL range in control subjects, are identified as well.

Thus, the phenotype of low adenosine syncope could be identified in 19% and that of high adenosine syncope in 18% of the whole syncope population (Figure 2). The clinical features of patients with low, normal, and high ABL are shown in Table 2. The clinical differences were modest, not reaching the level of significance.

DISCUSSION

The main findings of the study are that rest ABL, measured with the rapid “blot spot” method on whole blood, can distinguish a general population of patients with suspected NMS from healthy subjects. Among an unselected NMS population, two groups of patients with low and high outlier ABL values can be identified, which account for 19% and 18% of the population, respectively. These results are consistent with those of previous studies performed with the standard method of high-performance liquid chromatography in plasma with stop solution (3, 4, 8, 9). The normal range of ABL observed with the “blot spot” method was similar to that of 0.40 to 0.78 µM observed with the chromatographic method in the laboratory of Biochemistry of Timone Hospital in 120 healthy subjects (10).

In our previous studies (3, 4, 8, 9), we showed that several patients with syncope without prodromes and normal heart had value of adenosine plasma levels below the 5th percentile.
of healthy subjects and that several patients with positive vasovagal response during tilt testing had values above the 95th percentile of healthy subjects. The result of the present study shows that such correlation is more difficult to be found in an unselected general population. In a recent trial (10), the efficacy of theophylline, an adenosine receptor antagonist, was similar in patients with low and high AP measured with the chromatographic method, suggesting a lack of correlation between adenosine and clinical effect. Thus, it seems that the identification of low-adenosine syncope and high-adenosine syncope is more effective when the dosage of adenosine is performed in well-selected population affected by typical severe forms of syncope without prodromes and normal heart (3, 10) or in patients with typical features of vasovagal syncope as was the case in the pivotal studies (3, 9).

In conclusion, in this study, we have validated a rapid method ("blot spot") for rapid, easy-to-perform, and cheap measurement of adenosine in the whole blood. The low and high ABL limits, as defined in this study, may help to define the purinergic profile of patients affected by NMS and can be used in future studies. Because the expression level of adenosine receptor is also crucial to interpret the relationship between the adenosinerergic system and NHS, further studies are necessary to explore...
notably the ratio $A_1 R$ or $A_{2A}R$ expression/adenosine level ratio.

**Limitation of the Study**

Many subjects with NMS were on antihypertensive treatment, which can modify plasma adenosine values. In previous studies, antihypertensive therapy was stopped at the time of adenosine blood sampling (8, 9, 11, 12). Furthermore, we did not ask our patients to stop coffee or tea before the study, although caffeine modifies the concentration of plasma adenosine (13) and makes the receptors more sensitive to the action of adenosine (14, 15) and thus may influence the occurrence of syncope. We did not take this step because we wanted to study the basal values of adenosine in the daily life of patients without changing their lifestyle. Similarly, tilt test was not performed in fasting state that could have influenced its result.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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**ETHICS STATEMENT**

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

**AUTHOR CONTRIBUTIONS**

AG: conceptualization, investigation, data curation, methodology, writing—original draft, and writing-review and editing. MB: conceptualization, formal analysis, methodology, writing—original draft, and writing-review and editing. MC and MG: adenosine measurement. FE: data collection. JD and GP: investigation and writing—review and editing. RG: conceptualization, investigation, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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