We do not recommend routine pretransplantation chemotherapy for patients who have received treatment in the past. Our recommendation to treat patients with impending immunosuppression (from human immunodeficiency virus infection, induction for organ transplant, or other etiologies) who have not previously received antitrypanosomal therapy should be interpreted in the context of our overall BII recommendation for *T cruzi*–infected adults up to age 50 years without advanced heart disease. The major objective of etiologic treatment in this setting is to prevent development or progression of cardiomyopathy.

Our intention in explicitly listing anticipated immunosuppression as a patient category for treatment was to lend additional weight to the recommendation since we assume that antitrypanosomal therapy would be better tolerated, and theoretically more effective, before immunosuppression develops or is induced. Although data are lacking on this point, a secondary theoretical benefit is a possible reduction in the risk of reactivation. Advanced renal, hepatic, or cardiac dysfunction would certainly complicate therapy and constitutes a contraindication.

We agree with Altclas et al that posttransplantation monitoring is indicated whether or not a course of antitrypanosomal treatment was completed prior to transplant. As stated in the Clinical Review, we recommend that treatment decisions be individualized for all adult patients with Chagas disease, balancing the potential benefit vs the risk of drug toxicity.

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RESEARCH LETTER

Plasma Anandamide Concentration and Pregnancy Outcome in Women With Threatened Miscarriage

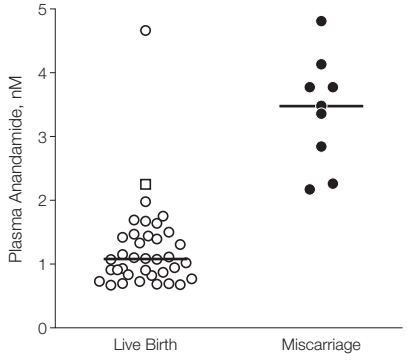
To the Editor: Approximately 40% to 50% of all human conceptions are lost before 20 weeks of gestation.[1] Recent animal studies suggest that the endocannabinoid anandamide (N-arachidonoyl-ethanolamine) is critical for both the synchronous development of the blastocyst and the endometrium in preparation for implantation, with low anandamide levels essential for successful implantation.[2] Plasma anandamide levels are regulated by fatty acid amide hydrolase (FAAH), the enzyme (up-regulated by progesterone) that metabolizes anandamide into arachidonic acid and ethanolamine.[3] Increased FAAH expression and lower anandamide levels have been demonstrated at the implantation site and low FAAH expression and high anandamide levels at the interimplantation site prior to successful implantation.[2] Levels of FAAH in peripheral blood mononuclear cells from uncomplicated early pregnancies were significantly lower in women who subsequently miscarried.[4] In women undergoing in vitro fertilization and embryo transfer, high plasma anandamide level at 6 weeks after embryo transfer was associated with failure to achieve an ongoing pregnancy.[5] We therefore investigated whether plasma anandamide levels could predict outcome in women presenting with threatened miscarriage.

Table. Characteristics of the Study Groups

Characteristic	Live-Birth Group (n = 36)	Miscarriage Group (n = 9)
Age, mean (SD), y	28 (1.5)	29 (1.6)
Body mass index, mean (SD)[a]	24.6 (2.4)	25.1 (1.9)
Gestational age at recruitment, mean (range)	8 wk (6 wk to 11 wk 1 d)	8 wk 1 d (6 wk 5 d to 10 wk)
Anandamide level, median (IQR), nM	1.065 (0.81-1.45)	3.47 (2.83-3.86)
Interval between recruitment and miscarriage, mean (range), d	NA	7.83 (5-14)
Gestation at delivery or miscarriage, mean (SD)	39 wk 1 d (1 wk 5 d)	8 wk (1 wk 1 d)
Birth weight, mean (SD), kg	3.40 (0.52)	NA

Abbreviations: IQR, interquartile range; NA, not applicable.
[a] Calculated as weight in kilograms divided by height in meters squared.

Figure. Plasma Anandamide Levels in Women With Threatened Miscarriage



The data are grouped according to those who proceeded to have a live birth and those who miscarried and are shown as individual cases. The median for each group is indicated by a horizontal solid line. The plasma anandamide levels were significantly different ($P<.001$; 2-tailed Mann-Whitney *U* test). Square indicates patient who developed severe preeclampsia and delivered at 33 weeks' gestation (birth weight of newborn, 1.85 kg).

Methods. Anandamide levels were measured in plasma from 45 healthy pregnant nonsmokers (TABLE) presenting to the early pregnancy assessment unit at 6 to 12 weeks' gestation with a threatened miscarriage (painless vaginal bleeding associated with a viable pregnancy). The assay was conducted with a high-performance liquid chromatography–mass spectrometry isotope dilution method (Waters Micromass Quattro Premier Mass Spectromer; Waters Corporation, Milford, Massachusetts).[6] The extraction and quantification were undertaken within 2 hours of blood collection. Patients, clinicians, and researchers were blinded to the results during the follow-up period.

Based on published anandamide data,[5,6] a minimum of 6 spontaneous miscarriages and 6 live births would allow a clinically significant difference of 40% in anandamide concentration to be observed with 80% power, assuming 2-sided $\alpha = .05$. Ethics committee approval for the study and written informed consent from each volunteer were obtained; participants did not receive financial compensation. Groups were compared using the Mann-Whitney *U* test; significance was set at 2-sided $P = .05$. Data were analyzed using InStat Version 3.01 (GraphPad Software Inc, San Diego, California), and the area under the receiver operating characteristic curve was calculated using Stata 9 (StataCorp, College Station, Texas).

Results. Of the 45 women, 9 subsequently miscarried and 36 had live births. Both groups had similar characteristics (Table) and underwent similar treatment. The median plasma anandamide concentration in the miscarriage group (3.47 nM; interquartile range, 2.83-3.86) was approximately 3-fold that in the live birth group (1.07 nM; interquartile range, 0.81-1.45; $P < .001$) (FIGURE). All women who miscarried had anandamide values greater than 2.0 nM; 34 of the 36 in the live birth group (94.4%) had anandamide values less than 2.0 nM. One of the 2 live-birth patients with values greater than 2.0 nM developed severe preeclampsia and delivered a 1.85-kg growth-restricted infant at 33 weeks; the other had an uncomplicated delivery of a 3.7-kg full-term infant. There were no distinguishing characteristics of outliers.

Using an anandamide level of 2.0 nM as an arbitrary cutoff point, a single plasma anandamide measurement provided a sensitivity of 100% (95% confidence interval [CI], 66.4%-100%) and a specificity of 94.4% (95% CI, 81.3%-99.3%) with a negative predictive value of 100% (95% CI, 66.4%-100%) and a positive predictive value of 82% (95% CI, 48.2%-97.7%) for subsequent miscarriage. The area under the receiver operating characteristic curve was 0.972 (95% CI, 0.882-0.999).

Comment. In this pilot study of women with threatened miscarriage, plasma anandamide level was associated with presence or absence of subsequent miscarriage. The study is limited by a small number of participants (with resultant wide confidence intervals) and requires replication in larger and more diverse populations.

Compared with tests based on peripheral blood mononuclear cells, anandamide-level measurement has an advantage of being based on whole blood and not requiring separation. If established as valid and clinically practical, anandamide measurement has the potential for improving the prediction and counseling of women presenting with threatened miscarriages.

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Study concept and design: Habayeb, Konje.
Acquisition of data: Habayeb, Finney, Evans.
Analysis and interpretation of data: Habayeb, Taylor, Evans, Konje.
Drafting of the manuscript: Habayeb, Taylor, Finney, Konje.
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Statistical analysis: Habayeb, Taylor.
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Study supervision: Taylor, Evans, Konje.
Financial Disclosures: Dr Konje reported having a patent (No. PCT/GB2005/050028) for the use of anandamide as a predictive test for preterm labor. PerkinElmer markets radiolabelled anandamide and produces a biochemical diagnostics test for chromosomal abnormalities in the first and second trimesters of pregnancy. PerkinElmer has first right of refusal for any patentable property emanating from Dr Konje's laboratory. No other disclosures were reported.
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