PREPRINT

# A more accurate method to interpret lactate dehydrogenase (LDH) isoenzymes' results in patients with uterine masses

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# Abstract

**Objective**: Since there are no approved reliable biomarkers for detecting preoperatively uterine sarcoma, lactate dehydrogenase (LDH) isoenzymes are usually required. We first evaluated the role of LDH isoenzymes in detecting uterine sarcoma risk, and then we tried to introduce a model to easily interpret the biochemical results.

**Methods**: We retrospectively retrieved records of patients who underwent surgical treatment for uterine masses from 2004 to 2016, and we compared data of 2211 patients with a definitive surgical diagnosis of uterine fibroids and 43 with uterine sarcomas. Quantitative relationships between serum LDH isoenzymes levels, as different single markers or in multiple assays, and the final diagnosis were investigated.

`Results`: LDH isoenzymes levels significantly differed between patients with benign uterine masses or sarcomas. LDH3 isoenzyme exhibited better predictive performances than the other four isoforms. Combining LDH3 with LDH1 isoenzymes into an inverse algebraic relationship, named Uterine mass Magna Graecia (U.M.G.) risk index, the accuracy of markers in discriminating between benign and suspicious malignant uterine masses was significantly enhanced, sensitivity at 100% and specificity at 99.6%, with nine false positive over 2211 benignant cases and no false negative over 43 sarcomas.

**Conclusions**: This retrospective analysis suggests to consider U.M.G. risk index as an inexpensive and accurate prognostic index that, once validated in a prospective study, may help clinicians in discriminating between benignant and suspicious malignant uterine masses.



**Keywords**: uterine fibroids; uterine sarcoma; U.M.G. risk index; lactate dehydrogenase (LDH) isoenzymes; fibroids morcellation


**Condensation**: The correct interpretation of LDH isoenzymes, through an easy to use risk-index, could help gynaecologists to safely choose the most appropriate treatment for patients with a uterine mass.

# 1 Introduction

Since the Food and Drug Administration (FDA) issued in 2014 a warning against the use of power morcellation (1), the risk of disseminating sarcoma, while morcellating uterine fibroids, has become a 'hot' topic (2,3). There is a re-emphasis on the extreme relevance to identify a preoperative way to estimate the risk of having sarcoma in women with presumed fibroids, especially because of the clinical impact that the absolute prohibition of resorting to minimally invasive surgery would have on women's health (4).

Moreover, given the availability of effective long-term medical treatments for women with symptomatic fibroids, clinicians should always obtain information about the sarcoma risk before to decide for a non-surgical approach for these masses (5,6).

Therefore, there is an urgent need for a tool useful to help gynaecologists

to assess sarcoma risk in patients with uterine masses and to determine the best therapeutic option in the subsequent decision-making process. Evidence suggests (7,8,9,10) that the serum levels of lactate dehydrogenase (LDH) and of some specific LDH isoenzymes (such as LDH3, 4 and 5) increase in patients with malignant tumours of the genital tracts and, particularly, with uterine sarcoma. However, neither the relationship between LDH levels and malignancy risk nor the interpretation of biochemical results (11) is completely clear in this group of patients.

With the aim to assess the value of LDH isoenzymes in determining the risk of uterine sarcomas in patients with a uterine mass, we retrospectively reviewed our data.

In particular, in order to evaluate the value of LDH isoenzyme in predicting sarcomas and to standardise the interpretation of the biochemical results, we assessed in our population the accuracy of each single LDH isoenzyme tested as individual prognostic index or of LDH isoenzymes used in a combined form (as a 'risk index') (12).

## 2 Materials and Methods

We retrospectively retrieved data from the whole charts of patients who underwent surgical treatment for uterine mass at our Institutions (Units of Obstetrics and Gynaecology, Azienda Ospedaliera Pugliese Ciaccio and Centro Oncologico di Eccellenza, Fondazione Tommaso Campanella, Magna Graecia University, Catanzaro, Italy) from January 2004 to February 2016.

According to our standard protocol, written consent to analyse data also for future clinical researches is always acquired from patients at the time of their hospitalisation. Moreover, approval was obtained from the institutional review board for reviewing and analysing data for this specific retrospective study.

To reduce the effect of confounding factors, we excluded from the analysis data the women with previous or concurrent cancer located at other sites, genetic susceptibility to gynaecological or non-gynaecological cancer (such as BRCA1-2 carriers, Associated Polyposis Conditions and patients with Fanconi syndrome), presence of an ovarian mass at the time of surgery, previous diagnosis of endometriosis and presence of cardiovascular and hepatic diseases, since all of these conditions could cause an increase in the serum levels of the assessed biomarkers. Based on the new uterine sarcoma classification system (13), carcinosarcoma cases were also excluded.

Among 3107 charts retrieved, 853 were excluded because either they did not satisfy the inclusion/exclusion criteria or their data were missing. The remaining 2254 patients were classified according to the final surgical diagnosis of uterine fibroids and uterine sarcomas (2211 vs. 43).

Values of LDH and LDH isoenzymes were reviewed from the chart and analysed according to the definitive surgical diagnosis. There are different ranges of normality for LDH and isoenzymes among different laboratories according to the used kits assay. However, this difference is really minuscule and without

significant difference. Usually, the lower and upper limits of total LDH are 135 IU/l and 214 IU/l, respectively, and the most common normal proportions of its isoenzymes are as follows: LDH1: 16.1-31.5%; LDH2: 29.2-41.6%; LDH3: 17.0-26.2%; LDH4: 5.9-12.3%; LDH5: 3.2-17.3% of the total LDH. The sum of LDH isoenzymes reaches 100% (14).

Other clinical (age, body mass index (BMI), parity), biochemical (Ca125, HE4) and imaging (mass diameters, vascularity) parameters were recorded and analysed. However, since neither CA125 and HE4 values nor imaging features were available for every single patient, they were not tested as predictive markers. Further, it was not part of the study's aim.

Statistical analyses were performed by the open source package R (15). The mean age for patients with fibroids and sarcomas was compared using Student's t-test. LDH isoenzymes levels between the benign and cancer patient groups were compared using the Wilcoxon rank-sum test (Mann-Whitney two-sample statistic).

Receiver operator characteristic (ROC) curves were constructed for each LDH isoenzyme and for the most accurate multiple assay biomarker (LDH3 and LDH1) that we identified [Uterine Mass Magna Graecia (U.M.G.) index] to differentiate between patients with sarcoma cancers and benign fibroids (16). For each ROC curve, the area under the curve (AUC-ROC) was compared using a non-parametric method accounting for the correlation induced (16). Bootstrap 99% confidence intervals (16) was performed to obtain the median sensitivities at set specificities of 98%, 99% and 99.5% for each LDH isoenzyme individually and for U.M.G. index. Discrete cutoffs for normal vs. elevated levels for markers were not used. For all statistical comparisons, a level $\alpha = 0.05$ was defined as statistically significant. To allow study reproducibility, dataset and R code are publicly available at `www.biostatisticaumg.it/umgrisk/`

# 3 Results

```
# To load into R the dataset from the website

www = "http://www.biostatisticaumg.it/umgrisk/magnagraecia.csv"
magnagraecia = read.csv(www, header = TRUE)
attach(magnagraecia)
```

The mean age for patients with fibroids was significantly lower compared to patients with malignant tumours (44.0 vs. 61.5 years respectively, $p < 0.001$). No significant difference was observed about parity ($2.6 \pm 0.9$ vs. $2.7 \pm 1.1$, $p = 0.47$) and BMI ($25.4 \pm 6.1$ vs. $26.2 \pm 4.3$, $p = 0.39$). The levels for all LDH isoenzyme tested differed significantly between subjects with uterine fibroids and uterine sarcoma (Table 1). Specifically, patients with uterine sarcomas exhibited significantly higher values of LDH3, LDH4 and LDH5 isoenzymes, and significantly lower values of LDH1 and LDH2 compared to the fibroids patients. Figure 1 shows LDH isoenzymes levels by the outcome.

```
# The mean ages for patients with fibroids and sarcomas
t.test(AGE ~ OUTCOME)

# LDH isoenzymes levels between the benign and cancer patients
wilcox.test(LDH1 ~ OUTCOME)
tapply(LDH1, OUTCOME, mean)
tapply(LDH1, OUTCOME, median)
wilcox.test(LDH2 ~ OUTCOME)
tapply(LDH2, OUTCOME, mean)
tapply(LDH2, OUTCOME, median)
wilcox.test(LDH3 ~ OUTCOME)
tapply(LDH3, OUTCOME, mean)
tapply(LDH3, OUTCOME, median)
wilcox.test(LDH4 ~ OUTCOME)
tapply(LDH4, OUTCOME, mean)
tapply(LDH4, OUTCOME, median)
wilcox.test(LDH5 ~ OUTCOME)
tapply(LDH5, OUTCOME, mean)
tapply(LDH5, OUTCOME, median)
library(lattice)
```

In a detailed exploratory analysis, all possible coupling of LDH isoenzymes was led (12). The multiple assays composed of LDH3 and LDH1 has turned out to be the best prognostic biomarker.

```
# unpublished Figure

par(mar=c(5.1,4.1,4.1,2.1), mfrow = c(1,2))
plot(LDH1, LDH3, col = "white", main = "LDH3 versus LDH1 in fibroids and sarcomas")
x = seq(from = 10, to = 40, by = 0.1)
y = 29 - 24/x
lines(x,y, lty = 4, col = "blue", lwd = 3)
points(LDH1[OUTCOME == "benignant"], LDH3[OUTCOME == "benignant"], pch = 21,
bg = "green", col = "darkgreen")
points(LDH1[OUTCOME == "malignant"], LDH3[OUTCOME == "malignant"], pch = 23,
bg = "red", col = "black")
text(25, 13, "green bullets: fibroids", col = "darkgreen")
text(17, 31, "red diamonds: sarcomas", col = "red")
text(16, 26, "blue line:", col = "blue")
text(16, 24, "LDH3 = 29 - 24/LDH1", col = "blue")
plot(LDH1, LDH3, col = "white", main = "U.M.G. risk index in fibroids and sarcomas")
x = seq(from = 10, to = 40, by = 0.1)
y = 29 - 24/x
lines(x,y, lty = 4, col = "blue", lwd = 3)
text(LDH1[OUTCOME == "malignant"], LDH3[OUTCOME == "malignant"],
```

```
UMG[OUTCOME == "malignant"], col = "red")
text(LDH1[OUTCOME == "benignant"], LDH3[OUTCOME == "benignant"],
UMG[OUTCOME == "benignant"], col = "darkgreen")
text(20, 34, "high-risk group", col = "red")
text(20, 14, "low-risk group", col = "darkgreen")
text(20, 13, "LDH3 + 24/LDH1 < 29", col = "darkgreen")
```

By means of recursive partitioning conditional inference framework as implemented in (17), the U.M.G. index had been defined as

$$U.M.G. = LDH3 + \frac{24}{LDH1}$$

yielding a very high significant ($p < 0.001$) partitioning with the value of 29 as a cut-point (12).

```
# unpublished Figure
# install.packages("party")
library(party)
model = ctree(OUTCOME ~ UMG)
plot(model)
```

In detail, the R code reported in `www.biostatisticaumg.it/umgrisk/` allows visualising two well-separated cloud points in the LDH3 versus LDH1 plane representing benignant versus malignant uterine masses, and the conditional tree representation. Table 2 exhibits the U.M.G. index performance as a classifier. With a 99.6% specificity (nine false positives over 2211 fibroids) and 100% sensitivity, 245.6 likelihood ratio for a positive result and 0.0 likelihood ratio for a negative result, U.M.G. index discloses its strong value (18) to rule in diagnosis, despite the different worldwide prevalence of fibroids and sarcomas.

```
# Table 2 accuracy of U.M.G. risk index
table(OUTCOME, UMG > 29)
(sensit = 43/43)
(specif = 2202/(2202+9))
(plr = sensit/(1-specif))
(nlr = (1-sensit)/specif)
```

Table 3 reports the AUC-ROC of LDH isoenzymes compared to U.M.G. index. The sensitivities at the pre-defined specificities of 98%, 99% and 99.5% were calculated for all markers individually and for U.M.G. index. Notably, the U.M.G. index achieved a sensitivity of 100% at the specificity of 99.5%, significantly ($p = 0.013$) improving LDH3 individual performance.

```
# set.seed is here for reproducibility purpose
# install.packages("pROC")
library(pROC)
set.seed(1234)
```

```
(roc1 = roc(OUTCOME ~ LDH1, auc=TRUE, ci=TRUE))
(roc2 = roc(OUTCOME ~ LDH2, auc=TRUE, ci=TRUE))
(roc3 = roc(OUTCOME ~ LDH3, auc=TRUE, ci=TRUE))
(roc4 = roc(OUTCOME ~ LDH4, auc=TRUE, ci=TRUE))
(roc5 = roc(OUTCOME ~ LDH5, auc=TRUE, ci=TRUE))
(rocumg = roc(OUTCOME ~ UMG, auc=TRUE, ci=TRUE))
# set.seed is here for reproducibility purpose
set.seed(1234)
roc.test(roc1, rocumg)
roc.test(roc2, rocumg)
roc.test(roc3, rocumg, method = "delong")
roc.test(roc4, rocumg, method = "delong")
roc.test(roc5, rocumg, method = "delong")
```

# 4 Comment

The chance to evaluate the risk of women affected by uterine masses of having a sarcoma is crucial for planning her best management approach. Until now, however, no reliable tools to determine this risk are available.

Uterine sarcomas are rare and aggressive mesenchymal tumours that originate in the smooth muscle of the uterus. They represent approximately 3% of all uterine neoplasms (19). The diagnosis of sarcoma is usually made after surgical treatment is performed in women in which a uterine fibroid is suspected. Therefore, the most appropriate surgical treatment is not always offered from the beginning. Since the rate of occult malignancy at the time of surgery for benign indications seems to be approximately 0.01-0.05% (20), the risk of uterine sarcoma should always be evaluated before starting long-term medical treatment or performing a conservative surgical treatment, above all if morcellation is expected. Indeed, it is widely accepted that patients with inadvertently morcellated occult uterine sarcoma have a poor prognosis (21,22). In April 2014, in a safety communication notice, the US FDA1 discouraged the use of laparoscopic power morcellation for the removal of the uterus or uterine fibroids because current evidence suggests that it poses a risk of spreading unsuspected and misdiagnosed cancerous tissue, notably uterine sarcomas, beyond the uterus. In reply to the FDA warning, some companies have recently withdrawn laparoscopic power morcellators. Some groups have proposed protected morcellation (23,24) while many others have always preferred the open surgical approach (25).

Despite these attempts to overcome the problem, there are several reasons due to which the exclusion of the risk of uterine sarcoma in women with uterine masses is crucial.

First, protected morcellation, which was recently suggested to minimise the risk of inadvertent tissue spread (23,26), could not be considered completely oncologically safe. It was observed that not only electromechanical power morcellation but also other 'manipulations' of the sarcoma, such as myomectomy

by even open surgery or hysteroscopic resection of submucous fibroids, are associated with increased risk and may affect survival, potentially due to upstaging (26).

Furthermore, products for protected morcellation require additional training and skill and may add operating time (27). Moreover, after the FDA communication, the utilisation of laparoscopy decreased. More than two-thirds of minimally invasive surgeons declared that they had changed their surgical approach planning after the FDA warning (28). Although utilisation of abdominal hysterectomy may decrease the specific risk of dissemination of occult malignancy, open procedures increase the surgical morbidity risk for patients (6).

Evidence (29,30) has recently shown that although there were more deaths from leiomyosarcoma after laparoscopic hysterectomy (86 vs. 71%), there were more hysterectomy-related deaths with the abdominal approach (32 vs. 12 %).

Finally, the possibility to exclude the risk of sarcoma preoperatively could be even more advantageous today, given the long-term medical strategies available to avoid unnecessary surgery in patients who would not benefit from surgical treatment, such as those affected from benign fibroids close to menopause (31).

In 1969, Widy-Kierska et al. (7) suggested that elevated serum LDH levels resulted in different gynaecological malignancies and that the over-expression of serum LDH3, 4 and 5 isoenzymes seemed to be specifically related to uterine sarcoma (6). The LDH enzyme catalyses the reversible transformation of pyruvate to lactate under anaerobic conditions, coupled with the oxidation of nicotinamide adenine dinucleotide reduced (NADH) to NAD1. Normal tissues each have a distinct LDH activity pattern, depending on their functions, and the levels of LDH increase in response to tissue injury, necrosis, hypoxia, hemolysis, and myocardial infarction. LDHA plays a key role in regulating glycolysis by catalysing the final step of anaerobic glycolysis; therefore, its upregulation facilitates the efficiency of anaerobic glycolysis in tumour cells and reduces their dependency on oxygen (32). Other authors (10) later confirmed the high negative predictive value (NPV) of a normal value, in uterine sarcoma, of the same LDH isoenzymes, which is consistent with our results. LDH isoenzymes from 1 to 5 are fractions of the total LDH and are expressed as a percentage. LDH is a ubiquitous cytosolic enzyme present in all tissues, exhibiting origin and tissue-specific isoenzymatic pattern (33). Each isoenzyme is a tetramer composed of subunits coded by two different genes, designated as M (muscle) and H (heart) (7,8). These two polypeptides give rise to five possible tetrameric forms: H4 (LDH1), H3M (LDH2), H2M2 (LDH3), HM3 (LDH4), and M4 (LDH5). In malignant tumours of the genital and digestive tracts, there is a characteristic shift in LDH isoenzymes to a predominance of muscle LDH forms (LDH3, 4 and 5) coupled with a decrease in the normally predominant heart types (LDH1 and 2) (7,8).

Today, the interpretation of the biochemical results, as well as the question of the usefulness of LDH-activity estimation, for the judgement of tumour risk are still discussed and unclear.

Based on these observations, we reviewed our data with the aim to evaluate the role of LDH isoenzymes in detecting uterine sarcoma risk and to introduce

an easy model for the interpretation of biochemical results in the management of patients with uterine mass.

ROC curve analysis, allowed us to stratify patients into two-risk classes: the low-risk group (score $\leq 29$) and the high-risk group (score $> 29$). This simple classification could be helpful in clinical practice, once validated prospectively. The likelihood ratio of zero for a negative result suggests that U.M.G. index could allow for the preoperative identification of the proportion of patients (low-risk class) who are affected by benign uterine masses and who may undergo surgery using a laparoscopic (protected) morcellation technique, thereby offering the advantages of laparoscopy to women of reproductive age (given the lower rate of pelvic adhesions compared to the open approach). Moreover, in the low-risk group, patients may undergo medical treatment, such as Ulipristal Acetate (UPA) or Gonadotrophin-releasing hormone (GnRH) agonist, both before reproduction and as an accompaniment to menopause, permitting them to avoid a surgical approach.

The advantage of the U.M.G. index is twofold. Firstly, it requires the inexpensive evaluation of serum parameters (LDH1 and LDH3), which is performed at almost all laboratories. Secondly, a simple blood sample allows avoiding the unnecessary and expensive evaluations in low-risk patients that are needed, conversely, in women identified by their U.M.G. index as being 'at-risk'. The risk of sarcoma for patients in this class can reach 11%, and it could be unacceptable to allow laparoscopy with open morcellation or to choose a conservative medical treatment for a patient in this class. Once validated, moreover, U.M.G. index could be useful to provide information about the risk of having sarcoma during informed consent for patients with presumed fibroids, particularly for the patients who are candidates for long-term medical treatment (34).

Despite the high dimension of the collected data, our study is affected by some evident limitations. The first limitation is related to the retrospective nature of our study. Moreover, our dataset appears to be heavily unbalanced: 2211 uterine fibroids versus 43 uterine sarcomas are reported. However, such impairment is related to an un-modifiable epidemiological data which determines a considerable difference in prevalence of uterine fibroids and sarcomas within the population. For these reasons, prospective validation of the U.M.G. index is required, and a prospective multicenter study has been already planned. Further data could also clarify whether the difference of age in fibroids vs. sarcoma patients interact as a mediator or a confounder within typical LDH isoenzymes levels.

In conclusion, the present research identified an easy-to-use and inexpensive risk index that allowed us to stratify 2254 patients with uterine masses accurately, excluding the risk of sarcoma with 100% sensitivity. This risk index could help clinicians to interpret biochemical results of LDH isoenzymes, to choose the most appropriate management approach for each patient and to provide the sarcoma risk information during informed consent for patients with presumed fibroids, particularly for those patients who are not candidates for hysterectomy.

Because of the retrospective nature of this study, potential confounding fac-

tors may have been missed. Therefore, a prospective multi-centric study will be needed.

## 5 Conflict of interest

The authors report no conflict of interest.

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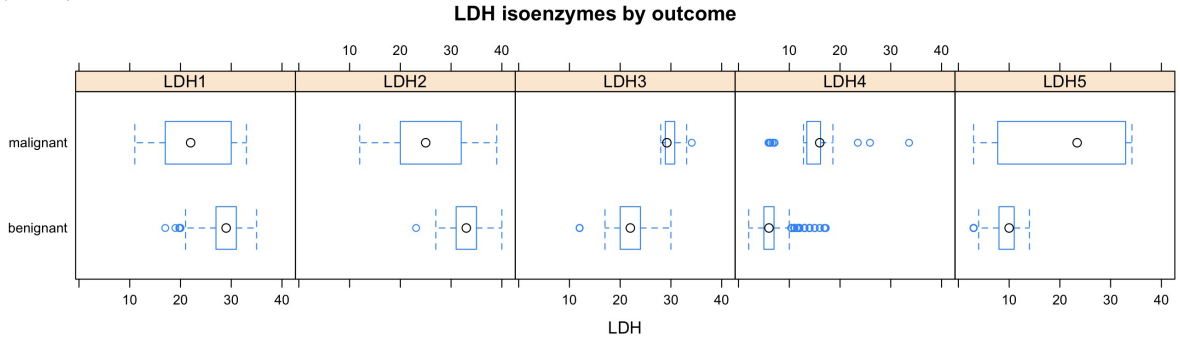


Figure 1. LDH isoenzymes by the outcome. LDH isoenzymes (1,2,3,4,5) values are shown in patients with benign and malignant uterine masses.

**Table 1: LDH iso-enzyme levels among patients with fibroids and sarcoma cancers**

| | Fibroids (2211) | Sarcoma (43) | p-value[(a)] |
|---|---|---|---|
| LDH1 | 29.0 (29) | 23.1 (23) | $p < 0.001$ |
| LDH2 | 33.2 (33) | 26.2 (25) | $p < 0.001$ |
| LDH3 | 22.2 (22) | 29.9 (29.2) | $p < 0.001$ |
| LDH4 | 6.3 (6) | 14.7 (16) | $p < 0.001$ |
| LDH5 | 9.3 (10) | 21.1 (23.4) | $p < 0.001$ |

[(a)] Wilcoxon rank sum (Mann-Whitney) test p-value.
Number are expressed as mean and median. Median is number between the brackets.
LDH: Lactate Dehydrogenase.

**Table 2. U.M.G. index performance as a classifier of uterine masses (fibroids and sarcomas), with 29 as a cut-point.**

| | U.M.G. ≤ 29 | U.M.G. > 29 | Total |
|---|---|---|---|
| Fibroids | 2202 | 9 | 2211 |
| Sarcomas | 0 | 43 | 43 |
| Total | 2202 | 52 | 2254 |

U.M.G. index = LDH3 + 24/LDH1
LDH: Lactate Dehydrogenase; U.M.G.= Uterine mass Magna Graecia.

**Table 3. The prognostic value of LDH isoenzymes and U.M.G. index in differentiating uterine fibroids from uterine sarcomas**

| **Biomarkers** | **Fibroids vs. Sarcomas** | | | | |
|---|---|---|---|---|---|
| | ROC-AUC (95% CI) | Cross validated sensitivity at: | | | p-value for comparison of ROC-AUC to U.M.G.[(a)] |
| | | 98% Specificity | 99% Specificity | 99.5% Specificity | |
| LDH1 | 71.8% (62.0-81.7) | 48.21% | 46.51% | 44.19% | $p < 0.001$[(b)] |
| LDH2 | 78.8% (69.6-87.9) | 52.21% | 51.16% | 51.16% | $p < 0.001$[(b)] |
| LDH3 | 99.8% (99.7-99.9) | 100.00% | 100.00% | 92.29% | $p = 0.013$[(c)] |
| LDH4 | 91.7% (86.8-96.7) | 79.07% | 74.42% | 72.09% | $p = 0.001$[(c)] |
| LDH5 | 73.0% (60.4-85.5) | 69.77% | 69.77% | 69.77% | $p < 0.001$[(c)] |
| U.M.G.[(a)] | 99.9% (99.8-100.0) | 100.00% | 100.00% | 100.00% | N/A |

[(a)] U.M.G. = LDH3 + (24 / LDH1). [(b)] Bootstrap method. [(c)] DeLong method.
LDH: Lactate Dehydrogenase; U.M.G.= Uterine mass Magna Graecia.