Open Access Journal of Oncology
ISSN: 2689-6168

Research Article *Volume 3 Issue 1*

Isoform A of Lactate Dehydrogenase (LDH-A), A Potential Ultra-Early, High Sensitivity Biomarker of Tumorigenesis

Gratacos EP[1]*, Oliver PG[2] and Gauto PA[2]

[1]Precision Metabolic Oncology Group, Universidad de la Habana, Argentina

[2]Center for Metabolic Therapy of Buenos Aires, Universidad de la Habana, Argentina

***Corresponding author:** Prieto Gratacos E, Precision Metabolic Oncology Group, Universidad de la Habana, Paraná 1073, Argentina, Tel: 0111540697478; Email: naturasenda@gmail.com

Received Date: August 20, 2020; **Published Date:** September 10, 2020

Abstract

A higher than normal percentual contribution of isoform A to the lactate dehydrogenase enzymatic cluster reveals a pathological shift towards fermentative metabolism somewhere within the organism of the host. The hypermetabolic phenotype expressed by tumour cells, a well-documented hallmark of cancer, is rooted on the catalytic action of several enzymes, amongst which hexokinase 2 and LDH-A are key players, supporting cell survival and neoangiogenesis as well as driving tumour growth. The physiological, intrinsic secretion of lactic dehydrogenase a in healthy humans has not been described formally and can be used as a functional frame of reference in the ultra-early detection of neoplastic transformation. In healthy subjects, even with total plasma LDH within the normal range, increases in the isoform a surpassing three or more standard deviations above its mean percentual contribution to the enzymatic cluster suggest a pathological reprogramming of energy metabolism. Herein, preliminary evidence is presented, supporting the notion of LDH-A as a screening tool for ultra-early, actionable detection of microtumours, during the initial or avascular phase of neoplastic progression.

Keywords: Isoenzyme A; Lactic Dehydrogenase; Warburg Effect; Metabolic Cancer Therapy

Abbreviations: RQ: Respiratory Quotient; ATP: Adenosine Triphosphate; Pfldh: Plasmodium Falciparum; LDH: Lactic Dehydrogenase; ELISA: Enzyme-Linked Immunosorbent Assay; Tb: Tumour-Bearing; Hs: Healthy Volunteers.

Preliminary Considerations

Depending on the relative concentration of each of its five isoenzymes, lactic dehydrogenase catalyses either the conversion of pyruvic acid into acetyl CoA or its fermentation into lactic acid, thus procuring an energy source for cells unable to extract it by means of oxidative phosphorylation. The implications are vast, and LDH-A´s fermentative power has been found to be an intricate part of survival mechanisms in many cancers, including breast, lung, prostate and pancreatic cancer. Fermentative hypermetabolism is a widely recognized hallmark of tumour cells. Evidence of ultrastructural mitochondrial pathology (cristodysmorphia) has recently been obtained by means of electron microscopy [1-9]. This work has provided visual confirmation as well as crucial insights towards a mechanistical explanation of the progressive deterioration of the respiratory quotient (RQ) in neoplastic cells. In vivo, the actual yield of oxidative phosphorylation is approximately 33.45 ATP/glucose, a remarkable efficiency in the harvesting of the metabolic power contained within high energy chemical bonds. Although not as high as the theoretical yield of 36 ATP moles for each mole of glucose sent through the glycolysis/OXPHOS oxidative

degradation cascade, the complete process of respiration is about sixteen-fold more efficient than fermentation alone. In the absence of properly functioning mitochondria, the energetic needs of anaplastic cells can only be met by low yield/high transaction volume metabolic pathways, such as anaerobic glycolysis, substrate-level phosphorylation and glutaminolysis. Though a striking biological regression from the evolutionary standpoint, fermentation provides a dependable, robust pathway to secure both building blocks and metabolically utilisable energy within anaplastic cells. Biological implications of plasma isoform and abnormalities. Initially isolated in Plasmodium falciparum (pfLDH) as well as in liver and muscle tissue (therefore dubbed "M", also known as LDH5), isoform A of the LDH cluster, provides an alternate pathway to harvesting the energy contained in pyruvate under conditions of overwhelming functional demand.

Besides providing a secondary, anaerobic energy source during intense physical exertion, this isoenzyme has no other known functions in healthy organisms. For properly rested individuals (LDH-A has a half-life of 9 hours), increases in plasma levels of this enzyme in excess of three standard deviations are almost certainly due to an increase in malignant fermentative metabolism taking place within anaplastic cells, due to a loss of their respiratory capacity [10-15]. For humans in good overall health, more specifically, individuals with no biochemically or clinically discernible tumoural pathology, it is a regular occurrence to find low -i.e. physiological- levels of several substances regarded as tumour markers. Based on our clinical experience and on general theoretical knowledge we set to consider as clinically significant –requiring further scrutiny- any increase of LDH-A greater than 2 standard deviations above the mean plasma concentration of healthy subjects. Such consideration stems from the fact that isoform A is found as a "constitutive secretion" in the blood of healthy individuals under 53 years of age in concentrations that rarely exceed 10 ng/ml. Immunohistochemical techniques have demonstrated that it is LDH-A -but not other isoenzymes within the family- that is predominantly expressed in neoplastic tissues. Isoform A of the LDH family can, therefore, be regarded as an early biomarker for highly glycolytic malignancies. Our group and many others have found elevated serum LDH-A in virtually all cancer patients tested, regardless of tissue origin, age, disease stage or previous treatment [16-22].

Eligibility Criteria for Healthy Subjects Providing Reference Values

Voluntary participants were required to be free of any apparent illness, without any history of previous oncological disease, and to be younger than 53 years of age. The upper cut-off value for total lactic dehydrogenase (LDH) was set at 200 U/L, placing every participant comfortably within the normal physiological range reported by regional laboratories. Subjects with a history of exertional myoglobinuria, hinting at an inborn error of the lactic dehydrogenase pathway, were also not included [23].

Patients, Materials and Methods

Thirty healthy volunteers, 18 females, 12 males, with ages ranging from 24 to 52 years (33, 32), were enrolled amongst healthcare professionals and software engineers to provide blood samples. Additionally, 28 patients with a confirmed diagnosis of cancer -in a spectrum of tumoral pathologies including cancers to the breast (21.4%), colon (14.3%), prostate (7.1%), uterus (10.7%), ovary (7.1%), liver (7.1%), lung (3.6%), kidney (3.6%), as well as exocrine pancreatic cancer (7.1%), glioblastoma (10.7%), sarcoma (3.6%), and chondroblastoma (3.6%)- were also analyzed for total LDH and the A fraction. For all participants, liver and kidney functions (Chemical Analyzer A15, BIOSYSTEMS), as well as hematopoietic status (Haematology Cell Counter Advia 560, SIEMENS), were analyzed in order to assess their overall physiological condition and establish a baseline. Blood specimens were obtained after eight hours of their last meal and twelve hours without any physical exertion. Participants were asked to arrive at our laboratory facilities by automobile, avoiding physicallydemanding means of locomotion such as climbing stairs, long walks or riding a bicycle. Throughout the process of acquiring the blood samples, technicians were careful not to strap the patients´ selected limb, nor allow them to forcefully make a fist to engorge the blood vessels. These common practices, aimed at improving accessibility to the veins in the upper extremities, are known to artificially increase total LDH in the sample. LDH-A was determined by means of Enzyme-Linked Immunosorbent Assay (Wuhan Fine Biotech, ELISA kit). Blood samples were heparinised and centrifuged at 1.600 rpm for 10 minutes, then processed according to manufacturer specifications. Written informed consent was obtained from each healthy volunteer and cancer patient. All participants and their close relatives were previously instructed in every instance on the necessary preparations and precautions.

Rationale for Age Exclusion Criteria

Cancer incidence in the population has been uniformly found to increase as a function of age. A similar trend has been demonstrated regarding all-cause mortality, with the probability of death doubling every eight years from puberty onward. First reported by mathematician and actuarian Benjamin Gompertz, this observation about the doubling time of the statistical probability of dying stands unchallenged today. Several authors have independently validated the Gompertz equation as a tool for modelling tumour growth [24-33]. Consequently, it stands to reason

that the probability of bearing an imperceptible, subclinical neoplastic pathology that could contribute to plasma LDH-A levels increases exponentially in direct proportion with chronological age. Limiting to 53 years the age of the healthy subjects included in the construction of a normal distribution for isoform A is intended to filter out individuals that could inadvertently carry an LDHA-secreting tumour, thus allowing for a moresensitive biomarker. This was a conscious decision on our part, intended to strongly enhance the sensitivity of the test, even at the expense of a marginal decrease in specificity.

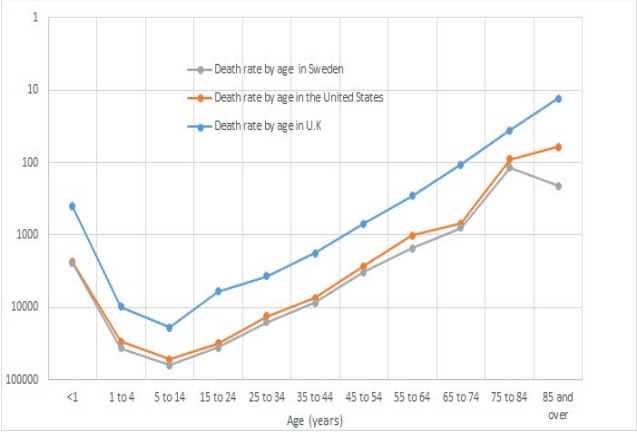

Figure 1: A demonstration of Gompertz´s law through a semi-log chart of all-cause mortality and age trajectories of Sweden, UK and USA populations (excerpted and combined from references 34, 35 and 36). Across many independent studies, the logarithm of the total death rate and the death rates for some individual diseases are linear functions of the chronological age, consistently doubling every ~ 8.4 years.

Results and Data Analysis

In our set of 30 healthy volunteers, plasma levels of LDH-A were found to range from 4.3 ng/ml to 10.0 ng/ml (6.84; 6.9; 1.48). The Confidence Interval 95% was estimated to be 6.39 -- 7.29. In this set of healthy subjects, the percentual contribution of isoform A to total LDH ranged from 1.7% to 4.8% (2.79, 0.7). Amongst the 28 cancer patients, LDH-A was universally elevated, ranging from 15.0 ng/ml to 51.1 ng/ml (30.8; 31.0; 6.07). Relative to the mean of healthy subjects, plasma levels of LDH-A were pronouncedly increased in all cancer patients. Furthermore, the percentual contribution of isoenzyme A to total LDH was also higher than the healthy mean (that is, 2.8 %) in 96.5% of the cases. However, patients N°27 and N°1 -whose percentual contributions fell within or in close vicinity to normal ranges- had unmistakably pathological total LDH levels (703 U/L and 912 U/L, respectively). On average, the percentual contribution of isoform A in tumour-bearing patients was 8,3% (2.8 -- 15.9).

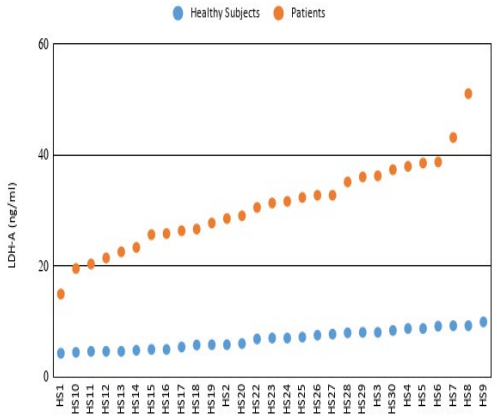

Figure 2: Isoenzyme LDH-A levels of 30 healthy subjects under 53 years of age (blue) and 28 cancer patients (orange), arrayed in ascending order.

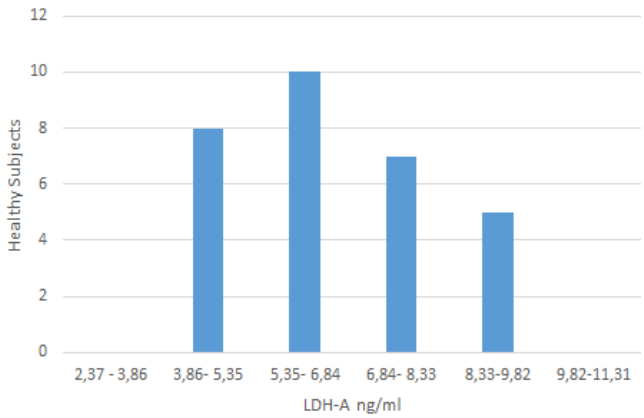

Figure 3: Distribution of isoenzyme LDH-A in healthy subjects (n=30) showing a rightward skew. Mean plasma level: 6.84 ng/ml, standard deviation: 1.48 ng/dl.

Healthy Subjects	LDH-A	LDH-A	Total LDH	Ratio Total/iso A	Percentual Contribution	Age	Gender
	(ng/ml)	(U/L)	(U/L)		%		
HS1	4,7	3,1	165,0	53,0	1,9	27	F
HS2	7,2	4,8	177,0	36,7	2,7	35	M
HS3	4,7	3,1	172,0	55,3	1,8	36	F
HS4	5,0	3,3	192,0	57,5	1,7	33	M
HS5	8,8	5,8	167,0	28,5	3,5	35	M
HS6	6,9	4,6	171,0	37,2	2,7	34	F
HS7	8,4	5,6	161,0	28,6	3,5	32	F
HS8	7,1	4,7	177,0	37,6	2,7	28	F
HS9	8,8	5,8	136,0	23,2	4,3	30	M
HS10	9,3	6,2	143,0	23,1	4,3	35	M
HS11	5,8	3,8	135,0	35,1	2,8	27	F
HS12	7,6	5,0	138,0	27,6	3,6	26	F
HS13	9,2	6,1	128,0	21,1	4,8	30	M
HS14	5,5	3,6	145,0	40,2	2,5	27	F
HS15	5,9	3,9	139,0	35,9	2,8	25	M
HS16	4,7	3,1	141,0	45,7	2,2	25	M
HS17	6,1	4,0	173,0	43,1	2,3	26	M
HS18	8,0	5,3	167,0	31,6	3,2	26	F
HS19	4,9	3,2	154,0	48,0	2,1	25	F
HS20	5,0	3,3	171,0	51,6	1,9	33	F
HS21	7,8	5,1	187,0	36,4	2,7	19	F
HS22	5,9	3,9	168,0	43,3	2,3	26	F
HS23	4,3	2,9	163,0	57,0	1,8	25	F
HS24	7,1	4,7	129,0	27,4	3,7	43	F
HS25	8,1	9,6	214,0	22,2	4,5	52	M
HS26	6,6	4,4	191,0	43,7	2,3	43	M
HS27	10,0	6,6	169,0	0,7	3,9	45	F
HS28	9,3	6,2	159,0	25,7	3,9	41	F
HS29	4,5	3,0	141,0	47,0	2,1	52	M
HS30	8,1	5,3	156,0	29,2	3,4	51	F

Table 1: Total LDH, isoenzyme A and percentual contribution of iso-A to total enzymatic cluster amongst healthy subjects (n=30).

Patients	LDH-A	LDH-A	Total LDH	Ratio Total/iso A	Percentual Contribution	Age	Subtype	Gender
	(ng/ml)	(U/L)	(U/L)		%			
Patient 1	38,8	25,8	912,0	35,3	2,8	59,0	Colon	M
Patient 2	32,8	21,8	335,0	15,4	6,5	62,0	Liver	M
Patient 3	27,8	18,4	373,0	20,3	4,9	57,0	Breast	F
Patient 4	19,6	12,9	159,0	12,3	8,1	63,0	Lungs	F
Patient 5	26,4	17,4	300,0	17,2	5,8	55,0	Breast	F
Patient 6	20,4	13,4	177,0	13,2	7,6	40,0	Breast	F
Patient 7	25,4	16,8	197,0	11,8	8,5	48,0	Breast	F
Patient 8	22,6	14,9	260,0	17,4	5,7	65,0	Prostate	F
Patient 9	31,7	20,9	252,0	12,1	8,3	65,0	Colon	F
Patient 10	26,7	17,6	265,0	15,1	6,6	38,0	Ovary	F
Patient 11	51,1	34,0	214,0	6,3	15,9	75,0	Sarcoma	F
Patient 12	29,5	19,6	250,0	12,7	7,9	49,0	Uterus	F
Patient 13	37,4	24,9	214,0	8,6	11,6	62,0	Kidney	F
Patient 14	35,2	23,4	209,0	8,9	11,2	61,0	Prostate	F
Patient 15	43,2	28,8	187,0	6,5	15,4	36,0	Breast	F
Patient 16	32,4	21,6	357,0	16,5	6,0	35,0	Ovary	F
Patient 17	28,6	19,1	220,0	11,5	8,7	58,0	Chondrobl	F
Patient 18	32,8	21,8	182,0	8,3	12,0	75,0	Glioblast	M
Patient 19	36,1	24,0	258,0	10,7	9,3	45,0	Uterus	F
Patient 20	38,6	25,7	391,0	15,2	6,6	64,0	Pancreas	F
Patient 21	30,6	20,4	275,0	13,5	7,4	47,0	Uterus	M
Patient 22	31,4	20,9	373,0	17,8	5,6	42,0	Liver	F
Patient 23	25,9	17,3	186,0	10,8	9,3	49,0	Glioblast	M
Patient 24	36,3	24,2	162,0	6,7	14,9	56,0	Colon	F
Patient 25	15,0	10,0	168,0	16,8	6,0	60,0	Prostate	F
Patient 26	38,0	25,3	439,0	17,3	5,8	45,0	Breast	F
Patient 27	25,7	17,1	703,0	41,1	2,4	65,0	Colon	F
Patient 28	21,5	14,3	179,0	12,5	8,0	41,0	Pancreas	F

Table 2: Total LDH, isoform A and percentual contribution of iso-A to total enzymatic cluster amongst patients with a confirmed diagnosis of tumoral pathology (n=28).

	Hs	tb	Units
N	30	29	
age	33.1	54.5	Yrs
Total LDH	192.0	287.8	U/L
Iso A	6.84	30.9	ng/ml
percentual contribution	2.93	8.3	%
	1.48	7.66	ng/ml

Table 3: Mean age, total LDH, isoform A and percentual contribution of iso-A to total LDH cluster for 30 healthy volunteers (hs) and 28 tumour-bearing patients (tb). , denotes the standard deviation of isoform A for each group.

Discussion

Given the fact that LDH-A can specifically enable fermentative neoplastic metabolism, plasma levels of this isoenzyme can conceivably increase in apparently healthy subjects going through the process of developing a microtumour or avascular neoplastic lesion. This increase in the fractional contribution of the isoenzyme may take place without pushing total LDH beyond the formal upper limit of the reference range. Such increment implies a "silent" or clinically imperceptible shift towards aerobic glycolysis, a type of non-exertional fermentative metabolism highly specific of neoplastic cells. Pathological increments in aerobic glycolysis

can be thus detected, non-invasively, during the initial or subclinical phases of tumorigenesis Mitochondrial injury, age-related anaemia, and several other factors contributing to a deteriorating "installed capacity" for cellular respiration, progressively develop as a function of age in apparently healthy humans (37-40). A shift towards fermentative metabolism in order to meet functional demands -such as repeated or persistent infections, inflammation, tissue damage, toxaemia, etc.- increases the probability of tumorigenesis (41-43). Our findings in tumour bearing patients (tb) placed the mean percentual contribution of iso-A to total LDH at a distance exceeding 3 standard deviations () from the mean percentual contribution of iso-A to total LDH in healthy subjects (hs), for an effect size of 3.7 (as per the formulation $_{tb}$ - $_{hs}$ / $_{hs}$). An estimation of the magnitude of the increment of plasma LDH-A in tumour-bearing patients relative to healthy subjects seemed appropriate in this context (39). At the beginning of the study, we assumed that even at relatively low values of n (for both the set of healthy subjects providing reference values and that of tumour-bearing patients) an effect size higher than 1.2 in the scale provided in Table 5 would disprove the null hypothesis (H_0).

$_{tb}$ - $_{hs}$ / $_{hs}$	8.3 - 2.9	3.7

Table 4: Computation of the effect size using Glass' estimator delta (). The numerator consists of the difference between the mean percentual contributions of LDH-A for both groups, while the denominator is the standard deviation of the second, healthy group (x_{hs}).

Qualification	Effect size (Θ)	Sample size requirement
Extremely small	0.01	n~100.000
Small	0.20	n~10.000
Medium	0.50	n~5.000
Large	0.80	n~500
Very large	1.20	n~50
Extremely large	2.00	n~10

Table 5: A scale of effect sizes and corresponding sample size requirements to achieve statistical significance based on the work of Sawilowsky et al. Cohen et al. and Glass. In the present study, the effect size was 3.7 -well above the higher value on the scale- denoting an extremely strong effect and probably hinting at the need for a wider (in the sense of age distribution) sample of healthy subjects. Also, see Study Limitations [40-43].

Study Limitations

Given the high sensitivity achieved by extracting references values from healthy individuals under 53 yrs of age, this type of measurement of LDH-A would require adjunct analyses to pinpoint the exact organic location(s) of the suspected incipient microlesions. This study, designed as an early detection device, provides only the framework for the clinical assessment of incipient signs of pathological metabolism and should be followed up by further investigation on collateral testing that can mitigate any loss of specificity stemming from the sensitivity/specificity trade-off. Also, given that the resulting effect size is -quite literally- off the chart, a bigger and more representative population sample would be needed, ensuring higher certainty and robustness to our findings. Our preliminary measurements already show an uptrend in plasma LDH-A in positive correlation with the age of healthy subjects. The estimation of the effect size in subsequent samples would have to correct for age-related increments, independent from neoplastic pathology, within tumour-bearing patients.

Conclusions

LDH-A´s inherent specificity to neoplastic metabolism makes it a useful proxy for systemic deficiencies of oxidative phosphorylation and ultra-early cancer detection. Increments in the percentual contribution of isoform A to total plasma levels of lactic dehydrogenase (exceeding 2standard deviations above the mean healthy concentrations) reveal a pathological shift towards fermentative metabolism at some level of the organism. Regular, systematic measurements of the isoform A of LDH could, therefore, be used as an ultra-early biomarker of tumorigenesis.

Conflicts of Interest

As of this writing, the authors have no conflicts of interest, directly or indirectly, by ownership or by affiliation with any brand, company or institution.

References

1. Ping Miao, Shile Sheng, Xiaoguang Sun, Jianjun Liu, Gang Huang (2013) Lactate Dehydrogenase A in Cancer: A promising target for diagnosis and therapy. IUBMB LIFE 65(11): 901-910.

2. Suh SY, Ahn HY (2007) Lactate dehydrogenase as a prognostic factor for survival time of terminally ill cancer patients: a preliminary study. Eur J Cancer 43(6): 1051-1059.

3. Yangbo Feng, Yanlu Xiong, Tianyun Qiao, Xiaofei Li, Lintao Jia, et al. (2018) Lactate dehydrogenase A: A key

player in carcinogenesis and potential target in cancer therapy. Cancer Med 7(12): 6124-6136.

4. Koukourakis MI, Giatromanolaki A, Sivridis E, Gatter KC, Trarbach T, et al. (2011) Prognostic and predictive role of lactate dehydrogenase 5 expression in colorectal cancer patients treated with PTK787/ZK 222584 (vatalanib) antiangiogenic therapy. Clin Cancer Res 17(14): 4892-4900.

5. Rong Y, Wu W, Ni X, Kuang T, Jin, D, et al. (2013) Lactate dehydrogenase A is overexpressed in pancreatic cancer and promotes the growth of pancreatic cancer cells. Tumour Biol 34(3): 1523-1530.

6. Matthew G Vander Heiden, Lewis C Cantley, Craig B Thompson Science (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324(5930): 1029-1033.

7. Dang CV (2012) Links between metabolism and cancer. Genes Dev. 26(9): 877-890.

8. Hanahan D, Weinberg RA (2011 Hallmarks of cancer: the next generation. Cell 144(5): 646-674.

9. Gabriel J Arismendi-Morillo, Alan V Castellano-Ramirez (2008) Ultrastructural mitochondrial pathology in human astrocytic tumors: potentials implications pro-therapeutics strategies. J Electron Microsc 57(1): 33-39.

10. Shona A Mookerjee, Akos A Gerencser, David G Nicholls, Martin D Brand (2017) Quantifying intracellular rates of glycolytic and oxidative ATP production and consumption using extracellular flux measurements. J Biol Chem 292(17): 7189-7207.

11. Ritu Arora, David Schmitt, Balasubramanyam Karanam, Ming Tan, Clayton Yates, et al. (2015) Inhibition of the Warburg effect with a natural compound reveals a novel measurement for determining the metastatic potential of breast cancers. Oncotarget 6(2): 662-678.

12. Dania C Liemburg-Apers, Tom J J Schirris, Frans G M Russel, Peter H G M Willems, et al. (2015) Mitoenergetic Dysfunction Triggers a Rapid Compensatory Increase in Steady-State Glucose Flux. Biophys J 109(7): 1372-1386.

13. Maekawa M (1988) Lactate dehydrogenase isoenzymes. J Chromatogr 429: 373-398.

14. Goldman RD, Kaplan NO, Hall TC (1964) Lactic Dehydrogenase In Human Neoplastic Tissues. Cancer Res 24: 389-399.

15. Fantin VR, St-Pierre J, Leder P (2006) Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. Cancer Cell 9(6): 425-434.

16. Sharma S (2009) Tumor markers in clinical practice: General principles and guidelines. Indian J Med Paediatr Oncol 30(1): 1-8.

17. (2002) Tumor markers: Past, present, and future. Diamandis EP. In: Tumor markers: Physiology, pathobiology, technology, and clinical applications. AACC Press, Washington DC pp: 3-8.

18. Deepshikha Mishra, Debabrata Banerjee (2019) Lactate Dehydrogenases as Metabolic Link between Tumor and Stroma in the Tumor Microenvironment. Cancers (Basel) 12(4): 932.

19. (2017) Principia Metabolica: Fundamentos Científicos y Clínicos para una Terapia Metabólica del Cáncer. Prieto Gratacós cuartavía Trans 5: 69.

20. Valvona CJ, Fillmore HL, Nunn PB, Pilkington GJ (2016) The Regulation and Function of Lactate Dehydrogenase A: Therapeutic Potential in Brain Tumor. Brain Pathol 26(1): 3-17.

21. Koukourakis MI, Kakouratos C, Kalamida D, Bampali Z, Mavropoulou S, et al. (2016) Hypoxia-inducible proteins HIF1α and lactate dehydrogenase LDH5, key markers of anaerobic metabolism, relate with stem cell markers and poor post-radiotherapy outcome in bladder cancer. Int J Radiat Biol 92(7): 353-363.

22. Jiang W, Zhou F, Li N, Li Q, Wang L (2015) FOXM1-LDHA signaling promoted gastric cancer glycolytic phenotype and progression. Int J Clin Exp Pathol 8(6): 6756-6763.

23. Wiener Laboratorio (2000) Para la Determinación de lactato deshidrogenasa en suero, plasma y líquido cefalorraquídeo. pp: 1-9.

24. Araujo RP, McElwain DLS (2004) A history of the study of solid tumour growth: The contribution of mathematical modelling. Bull Math Biol 66(5): 1039-1091.

25. Dolejs J, Marešová P (2016) Onset of mortality increase with age and age trajectories of mortality from all diseases in the four Nordic countaries. Dovepress 12: 161-173.

26. Thomas BL Kirkwood (2015) Deciphering death: a commentary on Gompertz (1825) 'On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. Philos Trans R Soc Lond B Biol Sci 370(1666): 1-8.

27. Olshansky SJ (2010) The Law of Mortality Revisited: Interspecies Comparisons of Mortality. J Comp Pathol 142(Supp1): S4-9.

28. Economos AC (1982) Rate of Aging, Rate of Dying and the Mechanism of Mortality. Arch Gerontol Geriatr 1(1): 3-27.

29. Larry V Hedges, Ingram Olkin (1985) Statistical Methods for Meta-Analysis. Orlando.

30. Glass Gene V, (1996) Statistical Methods in Education & Psychology. Hopkins Kenneth D (Ed) 3rd (Edn.), University of Colorado, Boulder.

31. Bajzer MŽ, Marušić S, Vuk-Pavlović (1996) Conceptual frameworks for mathematical modeling of tumor growth dynamics. Mathematical and Computer Modelling 23(6): 31-46.

32. Yoichi Watanabe, Erik L Dahlman, Kevin Z Leder, Susanta K Hui (2016) A mathematical model of tumor growth and its response to single irradiation. Theor Biol Med Model 13(6): 1-20.

33. Bajzer Ž, Vuk-Pavlović S, Huzak M (1997) Mathematical Modeling of Tumor Growth Kinetics. A Survey of Models for Tumor-Immune System Dynamics pp: 89-133.

34. Fanny Janssen (2018) Advances in mortality forecasting. Genus 74: 21.

35. Pascariu, Marius (2018) Modelling and forecasting mortality. University of Southern Denmark Unit of Epidemiology, Biostatistics and Biodemography pp: 1-1154.

36. Bengtsson T, Keilman N (2014) Perspectives on Mortality Forecasting. Swedish National Social Insurance Board 1-101.

37. Reinhard Stauder, Swee Lay Thein Haematologica (2014) Anemia in the elderly: clinical implications and new therapeutic concepts. Haematologica 99: 1127-1130.

38. Culleton BF, Manns BJ, Zhang J, Tonelli M, Klarenbach S, et al. (2006) Impact of anemia on hospitalization and mortality in older adults. Blood 107(10): 3841-3846.

39. Thomas N Seyfried (2015) Cancer as a mitochondrial metabolic disease. Front Cell Dev Biol 3: 43.

40. Seyfried T N (2012) Mitochondria: the ultimate tumor suppressor. in "Cancer as a Metabolic Disease: On the Origin, Management, and Prevention of Cancer." Wiley Online Library pp: 195-205.

41. Jacob J Adashek, Shumei Kato, Scott M Lippman, Razelle Kurzrock (2020) The paradox of cancer genes in non-malignant conditions: implications for precision Medicine. Genome Medicine 12: 16.

42. Albert Szent-Gyorgyi (1977) The living state and cancer. Proc Natl Acad Sci 74(7): 2844-2847.

43. Marina Shaduri, Marc Bouchoucha (2013) Life-Cycling of Cancer: New Concept.