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ON SPECIFIC VS. NON-SPECIFIC ENZYMIC INTER-ACTIVITY IN ACUTE MYOCARDIAL INFARCTION

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The main objective of the is the multi linear inter-activity relationship (MLIAR) study of non-specific markers as alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) involved in myocardial infarction, the major importance in the diagnosis and prognostic evaluation of the causes and the evolution of ischemic heart disease, in relation with the specific cardiac enzymes creatine phospho-kinase – CK and the lactate-dehydrogenase isoenzymes – LDH. The results obtained through employing the pre- during- and post- clinical data for 50 men and women series of patiencint with over 5o years age reveal the in both case the multi-linear mixed specific-non-specific dependencies are responsible for acute myocardic infarction, yet with a more complex behavior for men's casuistic when the re-infarction is predicted especially by the LDH (specific) activity.

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1. Introduction

2.

Due to very high specificity for a particular enzyme preparation, enzymatic methods applied to the determination of metabolites in biological fluids have almost completely replaced the non-specific chemical methods. This requires, for example, a selection of insoluble substrates used to determine enzyme activity and an optimal adaptation of analytical methods.

At the forefront of chemical medicinal research stays the elucidation of the role of enzymes in the production of myocardial infarction and in the monitoring of the treatment with recovery protective effect. It is now known that there may be a painless or atypical pain myocardial infarction (AMI), as acute coronary accidents, without ST segment or Q waves on electrocardiogram; therefore the use of the biochemical markers remains as extremely useful [1]. An ideal marker must meet the following characteristics: (i) it should be found in high concentration in the myocardium; (ii) it should not be found in other tissues; (iii) it should be released quickly and completely after myocardial damage; (iv) it should have certain persistence in plasma.

Long time it was considered that the enzyme creatine phospho-kinase – CK, then creatine kinase muscle B CK-MB - and the ratio CK-MB/total CK, would be the most useful enzyme to diagnose an AMI, removing the glutamic oxalacetic transminase - GOT from the diagnostic practice or the lactate-dehydrogenase isoenzymes – LDH [2]. Over time there were established limits for the determination of CK-MB as follows [3, 4]: (i) CK-MB isn't excreted in the urine and elevated plasma levels occurred between 6 and 10 hours from the clinical onset of the disease (in

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the absence of thrombolysis in AMI); (ii) In the reperfusion after the AMI, CK-MB appears faster and has lower plasma levels.

On the other hand LDH is an enzyme found in nearly all human tissues, the liver and skeletal muscle possessing the highest activity, while the heart muscle is having fewer enzymes [5]. This dehydrogenase is present in erythrocytes, kidney, lung, pancreas and brain. Normal human sera contain small amounts of LDH due to the normal disintegration of the tissue integrity, but the enzyme activity increases significantly in diseases with destruction of these tissues.

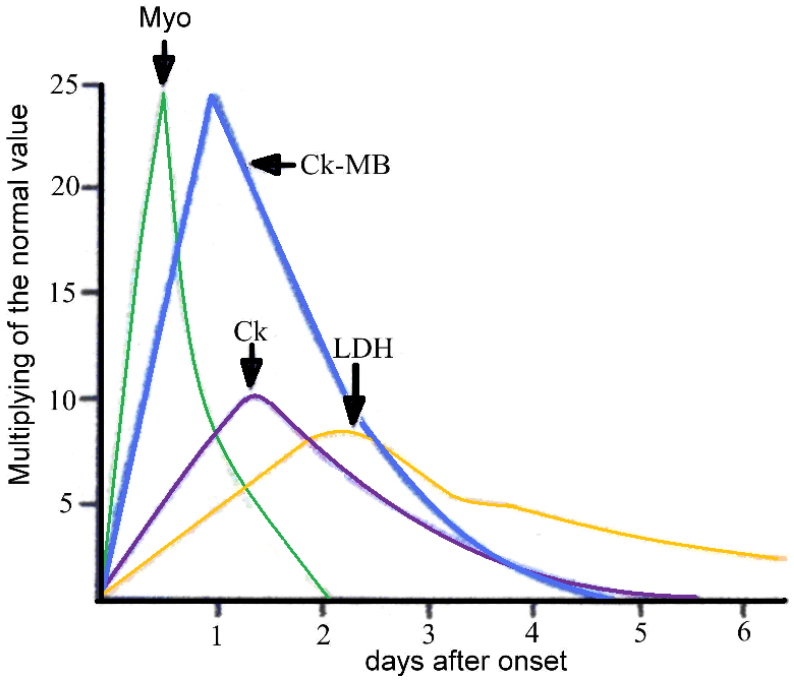

Fig. 1. CK, CK-MB, and LDH enzyme evolution in acute myocardial (Myo) infarction [6].

As a consequence the modeling of myocardial infarct in terms of LDH or even by simple enzyme CK evolution seems not enough for monitoring the acute myocardial attack; instead, as revealed in Figure 1, the creatine kinase muscle B (CK-MB) should be assumed as the main indicator for the phenomena; moreover it has to be investigated against the non-specific or less specific enzyme markers that eventually will replaced the fashioned LDH influence in cardiac muscle disease. The present work makes such a step forward by using the monitoring of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) as the complementary non-specific markers measured on a wide range of man and women patients either before during or after myocardial infarction occurrence.

2. Results and discussion

Aspartate aminotransferase (ASAT), formerly glutamate oxaloacetate transaminase, from necrotic cardiac myocytes, could be detected in the serum and could aid in the diagnosis of acute myocardial infarction (AMI). Today, we know that this enzyme is fully non-cardiospecific [7]; in particular, the lactate dehydrogenase (LDH) application in AMI diagnosis was described [8]; The first "cardiac" marker, i.e. the creatine kinase (CK) activity in serum, was described, emphasizing its rapid appearance while marking the associate increase in serum after AMI and its specificity for myocardial injury when compared with ASAT and LDH, without otherwise being able to suggest an effective methodology for its clinical application [9-12]. The discovery of the relatively higher concentration of creatine kinase muscle B (CK-MB) in the myocardium make it as the most important biomarker of cardiac injury, during which cardiology completely changed the diagnostic and therapeutic approaches to AMI, establishing new diagnostic goals for cardiac biomarkers other than retrospective confirmation of infarct, including early diagnosis in order to apply as soon as possible the new effective therapy for myocardium saving, i.e. the thrombolytic treatment [13-17].

On the other hand, high concentrations of alanine aminotransferase (ALAT) occur in the liver, and relatively low concentrations are found in the heart, muscle, and kidney. These enzymes are also used to monitor the course of treatment for hepatitis, active post necrotic cirrhosis, or the effects of drug treatment that might be toxic to the liver. In comparison to ASAT the ALAT test although being more specific for liver malfunction, due to its elevated level in myocardial infarction is to be investigated for the specific/non-specific character for acute heart attack.

As such Tables 1 and 2 the activities for the specific CK, CK-MB, and LDH and non-specific ALAT, ASAT enzymes are presented as clinically measured at beginnings (I), during (II), and at the end (III) of myocardial infarction for women and men over 50 years, respectively. The enzymic activity data are then in Tables 3 and 4 employed for emphasizing on the difference between the mono- and the multi- linear inter-activity correlation of the main myocardial CK-MB marker with the rest of AIM indices.

The analysis of the results in Table 3 reveal the women behavior on heart attack in terms of CK-MB=f(AIM indices) correlations:

- In all cases the higher correlations registered at the clinical entrance stage (I) is considerable decreased during the clinical observation (II) and slightly increase at the post-clinical stage (III) but considerably under those values quoted in stage –I;
- The non-specific ASAT indicator has the highest influence on CK-MB (myocardial infarction) action for mono-linear correlation, while its role is even more sustained by the bi-linear combination with ALAT, tri-linear combination with ALAT and CK, and quadric-linear dependency along the ALAT, CK and LDH enzyme activities.
- The higher inter-activities registered for ASAT mono- and multi-linear influential dependence in CK-MB biomarker is accompanied with maintained level of correlation in all subsequent II-for clinical, and III-post clinical stages.
- The lower inter-activity is registered at the LDH level, either for mono- as well as for bi- (along CK), and tri- (along CK and ASAT) enzymic linear dependence, showing therefore that the usually assumed specific enzyme has little role in assessing myocardial enzymic monitoring.

Overall, the over 50 year women hart attack resulted as being finely monitored by the aid of the hierarchy

ASAT> ALAT>CK>LDH

activities confirming this way the important place the non-specific enzymes in general and ASAT biomarker in special, have in modeling and preventing the myocardial activity either in pre-, acute- and post-stages of infarction.

Table 1: Enzymes activity [U/L] of ALAT, ASAT, CK, CK-MB, and LDH for women over 50 years at the beginnings (I), during (II), and at the end (III) of clinical action over myocardial infarction.

No.	**ALAT**			**ASAT**			**CK**			**CK-MB**			**LDH**		
	I	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**
1	21	26	31	64	153	191	89	210	483	17.3	53	150	792	1124	1170
2	30	42	81	37	77	276	83	889	4330	15.4	108.1	461	419	681	2220
3	17	37	38	49	190	240	113	292	325	29	66	75	865	1990	2529
4	64	85	1165	174	207	1035	1390	1439	1968	114	150.5	207	1345	1360	4580
5	77	280	375	86	430	570	462	1410	3470	14.4	89	267	847	1860	4580
6	27	31	39	31	104	117	187	750	1126	12	65.9	1126	665	887	1235
7	30	32	43	82	105	124	164	531	1625	23	44.1	133.1	1065	1092	1214
8	42	47	64	48	67	213	185	284	1759	29	60	162	430	1505	2140
9	41	51	69	29	140	152	53	691	1075	16	190	274.2	763	828	1112
10	16	59	100	17	228	644	40	960	3188	10	48	203	376	3512	4870
11	30	63	134	23	140	690	33	901	5180	10	64.2	422	356	4605	6500
12	37	71	87	52	221	393	150	887	2727	25.3	93	356	1281	1930	2035
13	33	34	35	61	63	99	1774	382	745	28	29.8	37	861	1008	1058

14	63	70	101	26	260	338	33	1922	2595	8	169	520	341	758	1292
15	43	80	93	54	330	582	77	1274	3720	12	162	648	1360	1945	2694
16	27	82	90	19	98	650	104	343	4125	14.5	23	360	1123	1612	3000
17	620	770	875	1075	1080	1180	10656	13000	19780	320	378	406.5	1915	2245	2290
18	28	40	51	16	40	162	62	142	2301	12.4	14.9	142	393	967	1157
19	41	90	109	46	242	249	36	1275	2105	8.1	140	227.1	615	1306	1980
20	39	43	92	34	47	411	180	350	5513	23.6	33.5	227	1266	1900	2577
21	43	85	101	33	38	52	35	54	76	13.8	20.2	28	1353	5820	7080
22	129	1145	1555	52	830	1590	44	293	554	23	32	39	544	1195	2475
23	23	23	45	19	27	73	98	136	479	13.2	23.6	45	850	1005	1730
24	22	23	25	55	60	63	2720	2730	3760	40	41	62	2307	2350	2580
25	43	46	51	41	43	47	157	160	178	27.7	28	29	1515	1576	1790
26	44	45	64	93	96	298	219	767	1940	37	105.1	204	320	570	738
27	29	54	62	30	228	256	195	1815	2025	26	102	105	470	800	1116
28	20	43	50	20	54	64	92	511	535	14.9	25	64	535	857	1310
29	17	22	26	21	153	224	75	1375	1910	22	140	211	295	446	676
30	28	63	85	18	60	92	20	338	1009	13	20	44	353	928	1640
31	29	47	54	17	86	159	45	747	1461	9.8	58	185	394	940	959
32	49	107	140	61	118	188	86	524	671	39.5	58	75.4	886	1610	1997
33	76	86	181	144	165	360	48	342	1370	11	51.6	129.8	827	2160	2620
34	15	16	22	17	35	77	57	225	402	6	10.7	27.6	562	837	1132
35	124	278	319	38	110	207	39	72	201	4	7	21	524	1134	1255
36	32	43	56	24	69	143	51	541	1134	14.7	44	126	554	1325	1695
37	78	95	111	301	430	576	1670	3280	6655	97.4	280	394	2540	2980	3980
38	31	258	1500	20	63	975	59	86	149	8.9	13	17	448	620	2035
39	37	44	57	34	90	117	209	620	1096	23.1	46	72	604	1320	1898
40	36	52	104	59	76	181	111	169	190	21	24	30	1380	1771	1868
41	52	73	81	156	134	316	1135	2725	2871	86.8	262	269.4	485	621	621
42	32	83	122	41	102	375	50	207	2322	9	23	164	574	1655	3755
43	25	54	58	38	129	246	53	725	2179	12	82	255	508	1299	1528
44	41	50	57	54	61	68	7190	8300	9430	22.3	43.3	55	737	841	1029
45	62	65	70	44	95	101	46	200	345	10	22	39	785	920	1264
46	12	38	84	20	23	315	79	1781	2925	11	90	162	542	827	1037
47	80	81	96	69	121	202	797	710	5424	40.6	2280	235	1245	1840	2110
48	42	44	97	58	77	100	78	708	953	7	39	48	237	1067	1175
49	38	65	76	80	92	580	93	1056	2744	18	189	246	272	491	2883
50	12	26	54	91	210	360	366	2165	3183	26	186	266	691	734	810

Table 2: The same record as in Table 1, here for men over 50 years.

No.	**ALAT**			**ASAT**			**CK**			**CK-MB**			**LDH**		
	I	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**
1	42	78	85	154	160	427	709	1770	2982	57	158	195	852	1819	2810
2	18	32	45	43	125	222	587	8200	12050	43.7	200	303	652	1880	3220
3	37	42	57	56	203	239	54	713	1440	20.6	99	247	1544	1557	2135
4	98	120	165	102	130	240	87	284	580	8.9	22	55	578	1160	1490
5	64	64	69	129	134	153	2016	2296	3054	49	50	60	823	849	855
6	22	262	327	15	349	550	96	735	2850	9.1	59	233	249	2520	2875
7	19	85	130	23	140	407	25	1034	1299	10	60	130	399	3960	4340
8	30	48	51	27	197	201	79	1232	1911	20	92.7	201.2	759	1176	1478
9	49	83	91	88	245	384	565	1560	2620	29.2	98.1	212	1735	1775	2204
10	52	65	77	36	154	239	78	1425	3323	15	185	410	976	980	2049
11	18	119	238	44	405	510	469	1258	1311	1	217	228	138	1755	2049
12	22	28	32	34	111	113	165	787	1162	22	87	158	551	576	970
13	30	31	32	90	100	113	39	423	1162	13.7	89	158	237	423	970
14	71	77	83	62	79	142	198	353	753	19	29	70.2	1336	1346	1420

15	24	48	53	36	173	249	101	2465	3970	84	113	247.9	287	491	524
16	31	53	900	31	335	1100	58	2950	4055	18.9	200	473.9	890	2184	2217
17	65	800	800	79	710	710	80	2121	2121	17.8	91	91	1023	2080	2080
18	28	76	155	32	89	184	153	737	1153	14.6	64.2	93	396	847	1314
19	21	52	170	19	73	900	85	304	3700	14	22.4	491.4	1104	2388	5530
20	26	33	42	21	75	144	33	133	1438	9	10	138	317	412	1184
21	24	50	137	18	37	134	21	1390	2254	9	82.7	230	375	928	1696
22	12	144	183	15	56	73	36	474	854	10.5	30	34	685	1814	1968
23	42	50	56	47	102	128	657	830	2910	24	35	51	750	775	801
24	91	126	152	51	168	660	127	330	3895	17	45	187	970	180	2205
25	63	101	113	47	286	297	93	1530	2596	4	92	194	771	1730	2185
26	15	70	78	18	125	397	56	600	2245	12	54	174.6	523	2352	2495
27	700	2025	2905	708	1440	2450	2500	2800	4235	130	149.7	159	3100	2495	9150
28	8	24	40	10	31	132	236	473	1125	12.8	31	119	292	742	9150
29	14	129	171	31	250	418	41	1421	1488	10	156	265	533	2694	3616
30	30	47	55	19	81	157	118	827	1294	7.1	71	109	613	1372	1494
31	44	51	87	35	171	200	72	1033	1846	12	153	175	297	587	600
32	46	62	65	48	121	128	118	849	1187	11	74	108	460	758	964
33	4	22	32	7	88	99	399	2356	3104	4	57	88	594	60	620
34	85	996	1420	124	2510	6220	109	503	546	28	65	70.7	609	5136	6070
35	27	47	54	27	64	82	131	1406	1649	26	67	137.3	441	1005	1158
36	40	51	81	45	68	296	303	314	2740	42	55	432	487	1512	1578
37	27	66	98	21	63	83	119	729	917	93	211.4	316.2	506	1128	1170
38	59	4600	4600	13	5540	5540	143	650	650	10	45	45	1371	11300	11300
39	18	51	58	24	212	240	127	2405	2820	74	247	333.4	352	1495	1721
40	47	155	160	43	594	780	122	3177	6090	19.9	267.7	811.5	2276	3715	5382
41	71	131	169	52	475	730	21 0	7381	10350	21	427.5	1405	1543	6060	9620
42	33	41	46	31	64	89	312	561	987	36	36	71.1	1146	1146	1497
43	19	22	71	18	19	59	53	57	832	12.8	13	33	524	559	1163
44	16	18	21	31	46	53	64	131	183	48	122	168	726	1385	1656
45	56	75	127	33	88	127	87	213	1034	15	27	33	325	502	540
46	42	69	105	56	121	279	122	544	2184	14	33	147	276	425	431
47	38	40	52	108	170	182	705	710	713	106	115	129	534	720	1440
48	41	90	192	36	61	125	37	274	455	7	33.1	153.8	419	3158	5045
49	122	139	169	450	600	651	4520	4650	6140	373	378.9	490	1341	1458	1560
50	50	55	110	133	153	202	884	1625	2256	35.6	60	95	2545	2550	2619

Table 3. Interactivity correlations (QSAR equation and the correlation factor R) of the CK-MB enzyme activity with other markers of Table 1 for the stages I-III (beginning, during and at the end) of clinical action.

Variables	Stage	R	Variables	Stage	R
CK	I	0.792	**LDH, ALAT**	I	0.905
	II	0.120		II	0.053
	III	0.316		III	0.147
LDH	I	0.495	**LDH, ASAT**	I	0.961
	II	0.043		II	0.115
	III	0.087		III	0.182
ALAT	I	0.881	**ALAT, ASAT**	I	0.962
	II	0.032		II	0.156
	III	0.099		III	0.478
ASAT	I	0.959	**CK, LDH, ALAT**	I	0.915
	II	0.112		II	0.126
	III	0.181		III	0.354
	I	0.813		I	0.962

CK, LDH	II	0.126	**CK, LDH, ASAT**	II	0.133
	III	0.319		III	0.323
CK, ALAT	I	0.900	**LDH, ALAT, ASAT**	I	0.963
	II	0.120		II	0.157
	III	0.347		III	0.485
CK, ASAT	I	0.960	**CK, ALAT, ASAT**	I	0.963
	II	0.130		II	0.159
	III	0.322		III	0.492
CK, LDH, ALAT, ASAT				I	0.964
				II	0.160
				III	0.497

Table 4: The same as in Table 3, here for men over 50 years against the values of Table 2.

Variables	Stage	R	Variables	Stage	R
CK	I	0.868	**LDH, ALAT**	I	0.348
	II	0.742		II	0.379
	III	0.696		III	0.573
LDH	I	0.259	**LDH, ASAT**	I	0.702
	II	0.196		II	0.326
	III	0.357		III	0.477
ALAT	I	0.342	**ALAT, ASAT**	I	0.876
	II	0.055		II	0.247
	III	0.101		III	0.139
ASAT	I	0.679	**CK, LDH, ALAT**	I	0.877
	II	0.020		II	0.755
	III	0.027		III	0.773
CK, LDH	I	0.875	**CK, LDH, ASAT**	I	0.875
	II	0.743		II	0.746
	III	0.721		III	0.740
CK, ALAT	I	0.875	**LDH, ALAT, ASAT**	I	0.877
	II	0.743		II	0.383
	III	0.700		III	0.573
CK, ASAT	I	0.869	**CK, ALAT, ASAT**	I	0.896
	II	0.742		II	0.761
	III	0.696		III	0.706
CK, LDH, ALAT, ASAT				I	0.898
				II	0.763
				III	0.774

The situation is somehow more complicated for the men behavior, as the results of Table 4 indicate:

- In mono-linear dependency the CK indicator correlates at best with CK-MB activity, while for all multi-linear regressions the ALAT-ASAT presence is that responsible for best (and higher than in mono-linear case) modeling of preclinical (I) CK-MB behavior in Table 2.
- However, here the bi- and tri- linear combination of CK with less non-specific ALAT rather than with non-specific ASAT enzyme has the relevant influence in CK-MB modeling of the men's heart attack in pre-clinical (I) stages.
- Again, as in the women case, the LDH specific influence is that less important for modeling the men's heart attack.

- Instead, the clinical-II and post-clinical III stages display considerable different output that that registered for women; as such, in general, the multi-linear description of CK-MB shows not necessary a drastic decreasing of the stage I dependence in the clinical monitoring, and even with increasing value for LDH + ALAT combined monitoring that continue to increase also in the post-clinical stage III (that is the case also when LDH is monitored alone as well as when all biomarkers are considered in CK+ASAT+ALAT+LDH combination in the III post-clinical stage); this behavior may be explained by the combined specificity of involved enzymes towards the myocardium muscle activity. For the rest cases, the stage III registers lower values than those of stage I but not considerably lowered leaving with the idea the men's infarction is somehow more persistent and potentially dangerous despite the clinical II stage inference.

Overall, the over 50 year men hart attack resulted as being complex monitored by the aid of the hierarchy

$$(CK+ASAT+ALAT+LDH) > (CK+ALAT+ASAT) > (ALAT+ASAT) > CK$$

of combined inter-activities showing that more elaborated biomarker combination is considered as finely the CK-MB heart muscle enzyme activity may be modeled in all phases of acute myocardial infarct.

3. Conclusions

Modeling and preventing acute myocardial infarction based on current medical enzymic contents analysis, in urine serum for instance, may highly contribute to the improvement and prolonger of the human life, being therefore at the forefront of the actual medicinal chemical research. Yet, the main problem is the selection of the enzymes to be analyzed such that they best parallel the myocardic acute muscle disease. Recently both specific less specific enzymes have been found as the appropriate markers for such endeavor, namely CK-MB, CK, ALAT, ASAT, among the fashioned LDH actions – see Figure 2 for both limited (left) and acute (right) myocardial infarction.

Yet, although in either cases CK and CK-(M)B activities appear as main indicators for acute infarctions symptoms, their inter-correlation with other involved enzymes remains somehow unclear so far. In this respect, the present contribution largely present the behavior of all these enzymes in pre-, during- and post- clinical monitoring of the cardiac symptoms such that comprehensive analysis is performed either for women and men subjects over 50 years.

The present results highly advocate that the case of acute myocardic infarction (AMI) in Fig. 2 –right is best modeled by the CK-MB multi-linear inter-activity relationships (MLIAR) primarily with CK, ASAT, and them with LDH, ASAT contributions to cardiac muscle's dysfunction – especially for men casuistic.

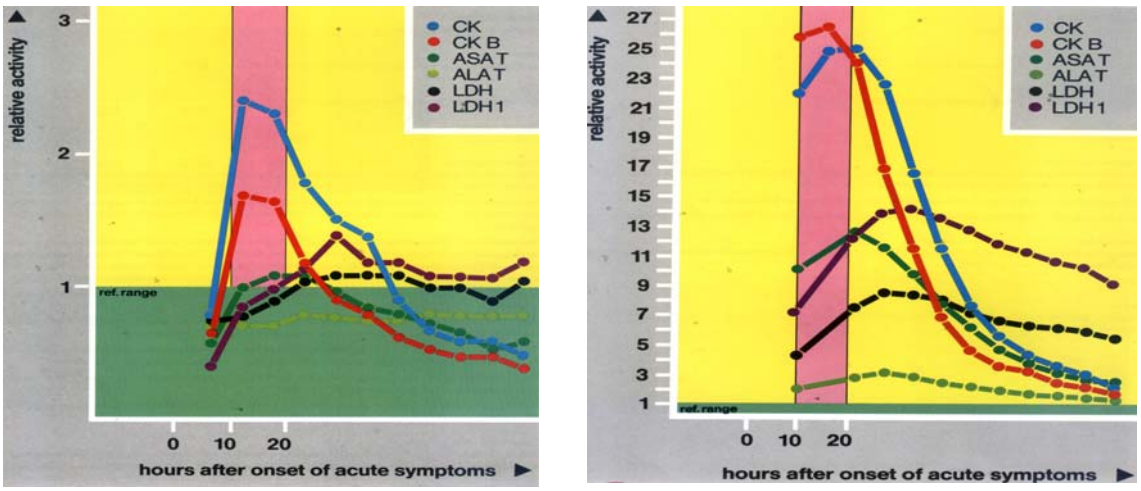

Fig. 2. Limited (in left panel) and acute (in right panel) myocardial infarction in terms f the CK, CK-(M)B, ALAT, ASAT and LDH enzymic activity before, within and post acute (clinical) symptoms [18].

Moreover, the present study asses that the women's behavior upon clinical treatment finely regulates the CK-MB inter-activity MLIAR dependencies with the other enzymes towards avoiding the post-clinical re-infarction that merely corresponds with the limited AIM casuistic of Fig. 2 –left.

Further studies, especially on the men casuistic, may provide that other non-specific eventually synergistically contribute to acute myocardic infarction, while their serum control may prevent it.

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