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Early Detection and Monitoring of Cancer with the Anti-Malignin Antibody Test

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ABSTRACT: The serum anti-malignin antibody (AMA) test determines the antibody to malignin, a 10,000-Da peptide present in patients with a wide variety of cancers.[1–6] A total of 3315 double-blind tests demonstrated that AMA is a general transformation antibody, elevated in active nonterminal cancer, regardless of the site or tissue type, with sensitivity and specificity of 95% on the first determination and >99% on repeat determinations.[7–9] Data have not however been published yet that indicate whether, in daily clinical practice, the AMA test provides accurate prospective and predictive information. Forty-two physicians from 11 states, who ordered the AMA test, performed blind, report here on their results on 208 determinations in the first consecutive 181 patients and controls. Used in monitoring treatment in 56 patients, the test predicted or agreed 94.1% overall with the clinical status. Used in early detection in 125 patients and controls, of which 118 now have confirmed diagnoses, AMA was elevated in 21, all of whom were proven to have cancer; AMA was normal in 97, none of whom had cancer. Transient elevated AMA occurred in 3%, followed by normal values. Seven patients with still uncertain diagnosis who have had elevated AMA on repeated tests for 1 year or longer include six who are symptomatic, and three whose families have a high frequency of cancer. The conditions of these 7 may include undetected cancer because of the 118 with now certain diagnosis the AMA test predicted all correctly. From our experience, the AMA test should be used together with other routine procedures whenever signs and symptoms suggest cancer to facilitate early detection.

KEY WORDS: anti-malignin antibody test, early detection, cancer, monitoring.

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I. INTRODUCTION

It is generally agreed that approximately 35% of people who die of cancer may be saved by earlier diagnosis and prompt treatment.[1] Screening programs for early detection of cancer are now common, privately sponsored as in the U.S., and under national health programs in the U.K. and elsewhere.

It might be expected on both theoretical grounds and from earlier data that a test that measures antibody would be useful in the early detection of cancer. On theoretical grounds, using the infectious disease model, antibody is more readily detected than antigen early in disease. Other serum tests for cancer, like CEA, because they measure antigen have been less reliable early but become more reliable late in disease as the tumor load increases and more antigen is released into the blood. In contrast, from earlier data on the AMA test,[7,8] (1) the presence of known cancer was closely correlated with elevated anti-malignin antibody from early stages throughout all but the terminal phase of the illness; and (2) the AMA test detected cancer in 2.3% of a group of 261 hospitalized medical-surgical patients who were not previously known to have cancer.

However, despite the extensive use in many clinical situations, and 3315 double-blind tests,[7-9] data have not been published indicating whether in fact, in routine clinical practice, the AMA test provides information early and accurately enough to be useful for detection and monitoring. The authors and participating physicians include 42 general practitioners, internists, surgeons, oncologists, and pathologists from 11 states, who report here their independent experiences as the first consecutive physicians to order the test for their patients. None were enlisted to participate in a study, nor were they aware that the results would be reported. Because there was a broad geographic and specialty distribution of the sample sources, and none of the physicians at one center knew those at the other centers or was aware of their experience until the data were brought together, and because the data were collected by similar methods, it was thought reasonable to pool the data in the hope that analysis would prove useful.

II. METHODS

A. Clinical Methods

1. Blind Determinations

The AMA test was performed by laboratory technologists who had no knowledge of the patient, except in cases #1 and #124 who were known to the technologist, and always blind to any clinical or histopathological data then available. In the *Early Detection* group, the histopathological findings were independent because they preceded the AMA data in 10 cases, or were obtained blind to the immunochemical data in the 22 cases in which the AMA data preceded the histopathology. The clinician did not influence the laboratory technologists because the clinician did not have the tissue diagnosis from the pathologist when the AMA test was performed in the other patients in the Early Detection group. The death rate, for designation of the *Terminal* state (see Section II.A.3), was by definition independent of the AMA test. The designation of *"no clinical evidence of disease"* by the clinician on follow-up was dependent on physical signs, biopsy, X-ray, CAT and MR scans, and other objective criteria and therefore unlikely to be influenced by serology.

2. Patient Selection

Tests were ordered to provide information relevant to early detection under two indications: (1) when signs and symptoms suggested cancer in the differential diagnosis, or (2) when the patient requested the test after hearing of its use with other patients. Some specimens also were submitted blind to the laboratory from healthy individuals as controls. The test also was ordered as an aid to management during the course of treatment, for example, to give information relevant to whether malignant cells remained after surgery, and to provide prognostic information (see *Monitoring* group in Table I).

TABLE I
Levels of AMA in 181 Patients and Controls with a Variety of Malignant and Nonmalignant Diseases

Case number, age, sex	Anti-malignin antibody level (μg/ml) (normal = 0–134) (elevated = >134)	Site, signs, and symptoms	Pathology: malignant (-stage) or benign	Comments	Physician
A. Early Detection					
1. Levels of AMA In Patients with Malignant Tumors					
1(DP)35F	377	Cervix, asymptomatic	Carcinoma-1	Cytology borderline, AMA borderline; 3 weeks later, cytology positive, AMA elevated; surgical removal; confirmed by pathology (see also #126 under *B. Monitoring*, and Table II)	W.R., J.T.
2(NR)50F	201	Cervix, asymptomatic	Carcinoma-1	Cytology positive; surgical removal; one focus <1 mm microinvasion and possible minimal lymph node involvement	W.J.T.
3(MS)85F	187	Cervix, asymptomatic	Carcinoma-2	Infiltrating vaginal wall	D.M., M.K.
4(SK)47F	299	Breast, asymptomatic	Carcinoma-1	1-cm lesion on mammography; biopsy infiltrating ductal carcinoma	H.D.
5(TB)60F	183	Breast	Carcinoma-1	0.6-cm lesion	W.R., J.T.
6(KH)31F	210	Breast, asymptomatic	Carcinoma-1	AMA 7 weeks postoperative	W.D.M.
7(JA)52F	245	Lung, asymptomatic	Carcinoma-1	First diagnosis and surgery 5 years earlier; asymptomatic at this time of elevated AMA; recurrence confirmed by X-ray, then surgery, well for a period; further recurrence, died 19 months later	A.H.L.
8(SR)59M	179	Lung	Carcinoma	Died 9 months later	P.G.
9(JWS)_M	192	Colon, bleeding	Carcinoma-1	Colonoscopy negative; after elevated anti-malignin, repeated colonoscopy found 3-cm carcinoma "hidden" under ileocecal fold; surgery	V.B.P.
10(SC)78F	380	Colon	Malignancy	Weight loss; weakness; 30 months prior surgery	V.B.P.
11(OS)80M	181	Prostate	Carcinoma-1	Alive 22 months later	P.G.
12(IF)_M	271	Bladder	Carcinoma		W.R., J.T.
13(ML)_M	210	Brain	Astrocytoma		W.R., J.T.
14(MP)72F	408	Brain	Glioblastoma	Alive 15 months later	P.G.
15(MH)31F	192	Brain	Astrocytoma	Alive 29 months later	P.G.
16(FW)86F	399	Skin	Carcinoma	Squamous cell cancer; died 9 months later	P.G.
17(JS)82M	302	Brain metastasis	?Primary lung	Died 3 months later	P.G.
18(DH)80F	361	Bone	Carcinoma	Metastatic; cancer of the breast 12 years earlier; died 8 months later	P.G.
19(MS)_F	160	Fever, anemia	Lymphoma		G.K., S.K.
20(NB)67M	275	Fever	Lymphoma	Bone marrow biopsy — lymphoma	W.P.
21(LR)_M	206	Hematologic	Leukemia	Acute lymphocytic	W.R., J.T.

N = 21; Mean = 256.5; SD ± 80.6

TABLE I (continued)
Levels of AMA in 181 Patients and Controls with a Variety of Malignant and Nonmalignant Diseases

Case number, age, sex	Anti-malignin antibody level (μg/ml) (normal = 0–134) (elevated = >134)	Site, signs, and symptoms	Pathology: malignant (-stage) or benign	Comments	Physician
2. Levels of AMA In Patients with Nonmalignant Diseases and Asymptomatic Healthy Normal Subjects					
a. Symptomatic Nonmalignant: Normal AMA					
22(UC)36F	56	Pelvic mass	Benign peritoneal cyst	Removed at surgery; confirmed by pathology	D.M., M.K.
23(SR)28F	9	Pelvic mass	Benign cystic leiomyoma	Removed at surgery; confirmed by pathology	D.M., M.K.
24(MP)28F	42	Pelvic mass	Benign cystic teratoma ovary	Removed at surgery; confirmed by pathology	D.M., M.K.
25(SS)69F	49	Pelvic mass	Benign cyst, ovary	Removed at surgery; confirmed by pathology	D.M., M.K.
26(SSt)50F	98	Pelvic mass	Benign cyst, ovary	Removed at surgery; confirmed by pathology	D.M., M.K.
27(EA)82F	52	Menopausal bleeding	Benign squamous metaplasia	Confirmed by pathology, endocervical atypia	D.M., M.K.
28(SV)40F	84	Endometriosis	Involutional	Confirmed by pathology, advanced endometrial	W.R., J.T.
29(AH)_M	129	Abdominal symptoms	Benign pancreatitis		D.M., M.K.
30(JD)60M	80	Abdominal symptoms	Benign pancreatitis	Enlarged head of pancreas on X-ray; initial clinical diagnosis of carcinoma; repeated normal AMA levels; healthy 8 years later	S.B.
31(JW)49M	71	Colon	Benign diverticulitis		T.P.
32(SP)73M	39	Colon polyp	Benign		D.M., M.K.
33(JC)78M	115	Heart disease	Hypertension	Chronic heart disease	W.J.
34(6678)46F	0	Hypertension	Hypertension	Benign	J.I.
35(007)	58	Hypertension	Hypertension		J.I.
36(HK)75M	58	Intestinal obstruction	Benign	Ileus with fecal impaction	M.M.
37(006)_	1	Ulcerative colitis, anemia	Benign		J.I.
38(RL)79M	69	Anemia, weight loss	Benign	Postcholecystectomy bile duct dilatation	V.B.P.
39(BP)42F	41	Allergic diathesis brain cyst	Benign	Initial test false positive; repeat normal	R.E.L.
40(301)41F	67	Severe dysplasia breasts	Benign		J.I.
41(EM)58M	110	Muscle atrophy	Benign		R.H.
42(Emc)62F	46	Bone mass	Benign	Pelvic mass on CT scan consistent with tumor or hematoma; open biopsy revealed normal repairing bone fracture	G.K., G.Go.
43(RE)36M	120	Vertebrae, pain	?Cancer, *in situ* ?reversed	Three elevated AMA (229, 407, 197) over 3 months accompanied by severe pain and sclerosing lesion of two vertebrae on CT scan; resolution accompanied by normal AMA	K.B.
44(BT)78M	i. 72 ii. 64	Depression	Benign	Marked weight loss, cancer suspected Regained weight on recovery from depression healthy 3 years later	W.J.T.
45(JGC)71F	51	Cancer-phobia	Benign		W.J.
46(EH)73F	0	Weight loss	Poor dentition	Initial test false positive (399); repeat normal	E.G.
47(WP)81M	—	Weight loss	Dysphagia	Initial test false positive (227); repeat normal	V.B.P., S.S.

N = 26; Mean = 58.4; SD ± 33.0

TABLE I (continued)
Levels of AMA in 181 Patients and Controls with a Variety of Malignant and Nonmalignant Diseases

b. Levels of AMA in Asymptomatic Healthy Normal Subjects

48 to 108	AMAS		AMAS		AMAS	
48(DD)M	20	49(CF)F	70	50(MJN)F	94	W.R., J.T.
51(ASH)F	71	52(MD)F	94	53(CK)F	54	
54(MS)F	120	55(MG)F	52	56(HF)F	33	
57(EG)F	18	58(LC)F	57	59(KS)F	41	
60(ET)F	91	61(JC)F	64	62(KL)F	58	
63(JT)M	74	64(OA)F	68	65(DG)M	106	
66(FA)M	11	64(FL)M	114	68(RH)F	48	
69(BB)F	17	70(NN)_	14	71(GM)M	70	
72(MG)F	70	73(ET)F	99	74(ST)F	66	
75(DL)F	34	76(FR)F	82	77(EB)M	85	
78(KW)F	118	79(OF)M	74	80(JR)F	24	
81(KE)F	71	82(CR)F	91	83(MK)F	54	
84(DB)F	56	85(RN)M	100	86(RL)F	130	
87(AD)F	83	88(EL)M	97	89(LM)F	70	
90(KD)F	97	91(LY)M	46	92(JT)F	96	
93(CG)F	133	94(MG)F	74	95(JB)F	87	
96(BC)	80	97(JM)M	87	98(AR)F	61	
99(DW)F	88	100(TF)F	84	101(PN)M	86	
102(JB)M	89	103(CP)F	97	104(AW)F	75	
105(AN)F	59	106(SG)M	91	107(SC)F	97	
108(JD)M	96					
109(NC,928)M		110(H,930)M		111(C,68)		D.M.
112(G,800)M		113(D,933)M		114(M,934)M		D.M.
115(S,935)F		116(C,950)M		117(P,951)		D.M.
118(RS)62M	70(Normal)	Normal		Initial test false positive; repeat normal		M.R.

N = 71; Mean Normal Asymptomatic AMA = 72.3 µg/ml; SD ± 28.9
a. + b. N = 97: Mean AMA levels in normal symptomatic and asymptomatic patients = 69.0 µg/ml; SD ± 30.9

Case number, age, sex	Anti-malignin antibody level (µg/ml) (normal = 0–134) (elevated = >134)	Site, signs, and symptoms	Pathology: malignant (-stage) or benign	Comments	Physician

3. Levels of AMA in Patients with Uncertain Clinical Diagnosis*

119(AB)60M	273	Gastric ulcer	Small prepyloric ulcer and erosive gastritis, both of which resolved; AMA values decreased over 1 year (299, 172, 193); not yet normal	B.H.Y., R.T.W.
120(KHT)32F	307	Thyroiditis	?Cancer *in situ*; high family cancer frequency (in 12 blood relatives); anti-thyroid microsomal antibodies elevated >700 units/ml; patient refused biopsy; AMA still elevated 2 and 3 years later (255 and 307 µg/ml)	J.L.M., S.C.F.
121(ss)38F	234	Breast, fibrocystic	?Occult cancer; high family cancer frequency; patient refused biopsy; AMA still elevated (211 µg/ml) 1 year later	P.Y.
122(DEM)58F	279	Breast, fibrocystic	?Occult cancer; high family cancer frequency; positive T-antigen test; patient refused biopsy	M.R.
123(CM)60M	242	Headache	?Occult cancer; chronic anxiety	R.S.
124(RL)65M	171	Prostatism	?Occult cancer; patient refused biopsy	M.R.
125(RR)64M	227	Pneumonitis	?Occult cancer; malnutrition; elevated CEA	G.G.

N = 7; Mean AMA level in patients with uncertain diagnosis = 247.6.; SD ± 40.8
*See **ADDED IN PROOF No. 2** with regard to "false-positive" AMA results that presaged clinical cancer.

TABLE I (continued)
Levels of AMA in 181 Patients and Controls with a Variety of Malignant and Nonmalignant Diseases

Case number, age, sex	Anti-malignin antibody level (μg/ml) (normal = 0–134) (elevated = >134)	Site, signs, and symptoms	Pathology: malignant (-stage) or benign	Comments	Physician
B. Monitoring: AMA Levels In Patients with Proven Malignant Tumors					
126(DP)35F	65	Cervix carcinoma, postoperative		3 months: No clinical evidence of residual disease (see also Table II for longitudinal follow-up with seven postoperative AMA determinations)	W.R., J.T.
127(WNR)50F	i. 130 ii. 89	Cervix carcinoma, postoperative		5 months: No clinical evidence of residual disease 6 months: No clinical evidence of residual disease	W.J.T.
128(MM)58F	179	Cervix carcinoma, metastatic		Squamous cell carcinoma in lymph nodes	D.M., M.K.
129(AK)68F	176	Cervix carcinoma, metastatic		Metastases to spine; died 7 months later	P.G.
130(EB)78F	124	Uterus sarcoma, terminal		Recurrence; metastases	D.M., M.K.
131(RS)73F	237	Uterus carcinoma, metastatic		Adenocarcinoma of peritoneum	D.M., M.K.
132(403)80F	172	Uterus carcinoma, postoperative		Clinical evidence of residual disease	J.I.
133(NB)48F	110	Uterus carcinoma, remission		No clinical evidence of disease 1 year later	P.G.
134(LL)59F	100	Ovary carcinoma, terminal		Clinical evidence of metastatic disease	I.P., R.W.
135(DG)54F	192(D)	Vulva carcinoma, postoperative		1 year: No clinical evidence of disease	J.M.W.
136(MC)_F	117	Breast carcinoma, postoperative		10 months: No clinical evidence of disease	M.P.
137(401)58F	77	Breast carcinoma, postoperative		4 years: No clinical evidence of disease	J.I.
138(402)66F	79	Breast carcinoma, postoperative		2 years: No clinical evidence of disease	J.I.
139(6568)58F	176	Breast carcinoma, postoperative		Clinical evidence of residual disease	J.I.
140(7268)57F	138	Breast carcinoma, postoperative		Clinical evidence of residual disease	J.I.
141(7311)47F	393	Breast carcinoma, postoperative		Clinical evidence of residual disease	J.I.
142(303)71F	145	Breast carcinoma, postoperative		Clinical evidence of residual disease	J.I.
143(306)44F	151	Breast carcinoma, postoperative		Clinical evidence of residual disease	J.I.
144(404)48F	165(D)	Breast carcinoma, postoperative		6 months: No clinical evidence of disease	J.I.
145(PT)55F	264	Breast carcinoma, postoperative		Clinical evidence of residual disease	D.J.F.
146(RW)72F	237(D)	Breast carcinoma, postoperative		3 months: No clinical evidence of disease	M.J.R.
147(PT)55F	i. 264 ii. 300	Breast carcinoma, postoperative		2 months: Clinical evidence of residual disease 8 months: Clinical evidence of residual disease	D.J.F.
148(405)60F	126	Breast carcinoma, terminal		Disseminated metastatic disease	J.I.
149(MS)70F	401	Breast carcinoma, metastatic		Chronic diarrhea; rising CEA	W.J.
150(RB)61F	175	Breast carcinoma, metastatic		Metastases to bone; died 4 months later	P.G.
151(RC)69F	81	Breast carcinoma, terminal		Metastatic to spine; died 11 months later	P.G.
152(CB)55F	371	Breast carcinoma, postoperative		2 months: Lobular carcinoma of other breast proven postoperatively	R.D.
153(HM)81M	97	Lung carcinoma, remission		Then alive with disease 21 months later	P.G.
154(5378)53M	189	Lung carcinoma, postoperative		Clinical evidence of residual disease	J.I.
155(MB)75M	204	Lung carcinoma, recurrent		Clinical evidence recurrent disease; died 9 months later	P.G.
156(KM)-F	347	Lung carcinoma, metastatic		Metastases to brain; died 4 months later	P.G.
157(MC)54F	370	Lung carcinoma, metastatic		Oat cell cancer; metastases to brain; died 5 months later	P.G.
158(BK)74F	262	Lung(?) carcinoma, metastatic		Metastases to skin; died 7 months later	P.G.
159(EM)79F	176	Lung carcinoma, metastatic		Metastases to spine	P.G.
160(AB)-F	97	Lung carcinoma, terminal		Died 4 months later	P.G.
161(305)75M	151	Laryngeal carcinoma		Clinical evidence of residual disease	J.I.
162(JG)61M	320	Esophageal Carcinoma, recurrent		Clinical evidence of residual disease Alive 20 months later	P.G.
163(DF)65M	125	Epiglottis carcinoma, remission		Alive 27 months later	P.G.
164(1539)76M	225	Colon carcinoma, postoperative		Clinical evidence of residual disease	J.I.
165(SLR)72F	201	Colon carcinoma, postoperative		Clinical evidence of residual disease	V.B.P.
166(ZH)72F	0	Colon carcinoma, postoperative		No clinical evidence of disease	K.O., G.G.
167(304)72M	65	Colon carcinoma, postoperative		1 year: No clinical evidence of disease	J.I.

TABLE I (continued)
Levels of AMA in 181 Patients and Controls with a Variety of Malignant and Nonmalignant Diseases

Case number, age, sex	Anti-malignin antibody level (μg/ml) (normal = 0–134) (elevated = >134)	Site, signs, and symptoms	Pathology: malignant (-stage) or benign	Comments	Physician
168(GR)55F	i. 160	Hepatic metastases, postoperative		4 months after left lobectomy for hepatic metastases (colectomy 1982 Duke's B2)	D.S.G.
	ii. 209			4.5 months postoperative	
	iii. 49			6 months postoperative	
				11 months postoperative; CT scan normal, no clinical evidence of disease	
				18 months postoperative: No clinical evidence of disease	
169(DH)63M	i. 211	Hepatic cholangiocarcinoma		Fever; recurrence 32 months, post-chemo- and radiation therapy	M.A.
	ii. 330			1 month after (i): local invasion	
170(EW)66F	46	Kidney carcinoma, remission		Abdominal, brain, and chest CT scan normal	G.G.
171(DH)53M	299(D)	Ureter carcinoma, postoperative		1 year: No clinical evidence of disease	H.D.
172(JH)83F	114	Bladder carcinoma, remission		25 months postoperative: No clinical evidence of residual disease	P.G.
173(SH)71M	102	Bladder carcinoma, terminal		Died 9 months later	P.G.
174(IF)_M	266	Bladder carcinoma		Biopsy	W.R., J.T.
175(RD)66M	45	Prostate carcinoma, remission		15 months postoperative; no clinical evidence of residual disease	P.G.
176(RD)61F	86	Brain astrocytoma, terminal		Died 2 months later	P.G.
177(EG)65F	108	Skin melanoma, terminal		Died <12 months later	P.G.
178(003)58F	206	Lymphoma, malignant		Clinical evidence of residual disease	J.I.
179(004)68F	140	Myeloproliferative disease		Clinical evidence of residual disease	J.I.
180(005)48F	362	Lymphoma, malignant		Clinical evidence of residual disease	J.I.
181(008)56M	51	Hodgkin's disease, remission		No clinical evidence of disease	J.I.

Abbreviations: Patient's initials or identifying code number are in parentheses in the first column of the table, physician's initials in the last column; "Normal" and "Elevated" anti-malignin antibody in serum (AMA) level — see Section II.B; "i, ii, iii" indicate different specimens from the same patient; "Remission" and "Terminal" — see Section II.A; "No clinical evidence of disease" and "Clinical evidence of disease" — independent blind clinical evaluations made at the same time as the AMA test was performed unless otherwise specified; "Later" — refers to time after the AMA test; number of months after surgery that the AMA test was performed is indicated before and after the word "Postoperative"; (D) — indicates disagreement between the elevated AMA test and the clinical evaluation that there was no clinical evidence of residual disease.

3. Clinical Criteria

The diagnosis and clinical evaluation were made in all but 7 of the 181 cases by surgery and histopathology, assisted by CAT and MR scans, by response to treatment, and by later clinical course. Of the 181 patients, all listed in Table I, in addition to the seven patients with *"uncertain diagnosis,"* only one of whom (#119) had a biopsy, the following medical disorder and healthy controls did not have surgery or biopsy, i.e., no histopathological diagnosis: #30, 33–41, 43–118. Histopathological and clinical stagings were conventional; all stages of cancer, from localized to diffuse metastatic disease, were represented. All patients in the *Monitoring* group had received or were receiving surgery, chemotherapy, radiation, or combinations thereof. The follow-up period to determine the false-positive and false-negative rates was a minimum of 1 year. In the *Monitoring* group, those cancer patients with normal AMA results were considered as false-negative until 1 year had elapsed; if dead within 1 year, they then were designated *Terminal Cancer* as in earlier studies.[7–9] Similarly, as in earlier studies, the correlation was examined between normal AMA levels in patients in remission, with clinically *"no evidence of disease."* Unusually long or short

survivals are noted separately in Table I. Each of the authors who were clinicians, as well as each of the participating physicians listed under Acknowledgments, was responsible only for their own patients for verifying the accuracy of the data in this communication, both the clinical data that were provided by them and the laboratory data that were received.

4. Collection and Delivery of Serum

Of the sera from 181 patients, 140 of 208 were shipped completely at random from 11 states overnight in dry ice to Oncolab, Boston; 68 of 208 sera were assayed at Baptist Hospital, Miami. Within 24 h of the specimen being drawn, the test was performed and the results reported to us. The test was determined only when the serum was collected and delivered under the following conditions previously found to minimize the loss of AMA, which is an IgM:[6] (1) blood was drawn in vacutainer tubes B.D. #6440 (which lack silicone coating on the walls of the tube) (purchased from Becton Dickenson); (2) the tube was the first-drawn if other tubes were being drawn; (3) serum separators were not used (absorb antibody); (4) the blood was centrifuged in the refrigerator or in a refrigerated centrifuge; (5) the serum was transferred by Pasteur pipette to a NUNC tissue culture tube (purchased from Thomas Scientific, Swedsboro, NJ) (minimal absorption of protein to walls); (6) the serum was frozen immediately in dry ice and shipped in dry ice overnight; and (7) the serum was received within 24 h of the blood being drawn. The accuracy of the test has been shown to be reduced in proportion to the time the serum is stored frozen longer than 24 h before determination.[9] Attention to these details is critical to the accuracy of the test.

During the period that 208 tests here reported were performed, 28 additional sera had to be excluded from analysis because they did not conform to the above-mentioned preset conditions: 5 because they arrived at the laboratory thawed, 10 because they arrived 48 h or longer after the blood was drawn, and 13 because they had been separated from blood cells by centrifugation at room temperature. In these excluded specimens, the diagnosis of the patients or controls from whom they originated is unknown. Other than these exclusions, the results here summarized represent the first tests consecutively performed on all the sera requisitioned in daily practice and shipped to the laboratories for which at least 1 year has elapsed for follow-up.

B. Laboratory Methods

The same quality-controlled procedure[10] used for all previous 3315 double-blind AMA tests[7-9] was used by both laboratories, characterized by specific immunoadsorption of the antibody from serum to TARGET® reagent (purchased from Brain Research, Boston). TARGET reagent consists of malignin bound covalently to bromoacetylcellulose;[1-4] in liquids it forms an insoluble particulate suspension. Malignin is a 10,000-Da peptide isolated from glioblastoma cells grown in tissue culture in a concentration of 0.1 to 1 mg/g wet weight of cells.[2] Malignin, isolated repeatedly over 20 years of tissue culture, is of constant composition, containing 89 amino acid residues including the characteristic high glutamic and aspartic acids and low histidine (13 glutamic acid, 9 aspartic acid, 2 histidine). An 0.2 ml amount of TARGET regeant is shaken vigorously with 0.2 ml of patient's serum at 0 to 5°C, washed with cold saline, then shaken vigorously with 0.25 *M* acetic acid at 37°C to elute the bound antibody, which is then quantitated as protein by adsorption at 280 μm and expressed as microgram per milliliter of serum.[10] Because malignin is covalently bound to bromoacetylcellulose to form TARGET reagent, dilute acetic acid does not elute malignin, but only elutes the specific antibody, AMA, which has been bound to malignin noncovalently. All specimens were determined in duplicate. Known amounts of monoclonal AMA[5,6] were assayed as positive controls for each serum. Two species of AMA, demonstrated to exist in human serum *in vivo* and produced and isolated *in vitro*,[5,6,10] are quantitated in two tubes for each serum determination: slow-binding (2-h reaction time), STAG — slow TARGET-attaching globulin (so named before it was known to be an IgM

antibody); and fast-binding (10-min reaction time), FTAG. One tube, placed in a shaker for 2 h, permits the FTAG to bind in the first 10 min, and STAG to bind the rest of the time. The second tube, agitated only 10 min, represents FTAG. The amount of FTAG is subtracted from the amount of STAG to give the Net TAG. The quantitative limits for designating normal and elevated results were the same as in earlier studies,[7-9] that is:

	STAG	FTAG	Net TAG	Interpretation
Range	100–399	50–299	0 to 99 µg/ml	*Normal*
	100–399	50–299	100 to 134 µg/ml	*Borderline* (if in same range, i.e., 100–134 when repeated, the result is *Normal*)
	100–399	50–299	>135 µg/ml	*Elevated*
	>400	Any value	Any value	*Elevated*
	Any value	>300	Any value	*Elevated*

If STAG and FTAG are both elevated, as for example if STAG is 500 and FTAG is 400, the Net TAG would be 100 and might be mistakenly accepted as normal if both the individual elevations of STAG and FTAG are not noted. Repeat determinations are routinely requested on all *Elevated* values.

III. RESULTS

The clinical and laboratory data for each patient are summarized in Table I. Although the specimens were received at random, that is, on any given day the nature and origin of the specimens were unknown, the results in Table I are organized into groups for the reader's convenience: (1) by the purpose of ordering the test, which was either early detection or monitoring; (2) by the AMA test results; (3) by the final diagnosis — benign or malignant; and (4) by the type of cancer. The initials in the last column are those of the author or participating physician, who was the responsible physician or reporting laboratory director in each case. There were 125 patients and controls in the *Early Detection* group, and 56 patients in the *Monitoring* group, comprising a total of 181 patients, for which a total of 208 tests were performed.

In Table I, Section A on *Early Detection*, the first 125 cases indicate the accuracy of the test in a wide range of cancers, which represented a cross section of different types of cancer that are likely to be encountered in practice. Where the diagnosis was confirmed, there were 21 of 21 correct positives (elevated AMA), and 97 of 97 correct negatives (normal AMA). In seven cases (5.6%), #119 through #125, the clinical diagnosis is still uncertain.

In both cancer and noncancer groups in the *Early Detection* group, the AMA test results were in agreement with the histopathological findings in all 32 cases where these findings were available. AMA test results preceded the histopathological examination in 22 of the 32 cases (case #1–5, 7–10, 19, 20, 22–28, 30, 31, 42, and 43), and followed the histopathology in 10 cases (#6, 11–18, 21, and 41). The number of patients found to have cancer out of the number selected on each clinical indication, that is, the yield, was as follows:

1. Where symptoms and signs suggested cancer in the differential diagnosis, 48 patients were tested, and of these, 18 cases of cancer (37.5%) were detected.
2. Where patients requested the test, ten patients were tested, and of these, two cases of cancer (20%) were detected.
3. Among 70 healthy controls tested, 1 case of cancer (1.4%) was detected.

Patients suspected of having cancer, who then had a normal AMA test, and a clinical diagnosis of noncancer are shown in cases #22 through #47. Cases #22 through 26, 28, 42, and 43 had a variety of abdominal and pelvic masses and AMA test results in the normal range, which were proven benign at biopsy or surgery. The other cases #22 through 47 had a variety of nonmalignant medical and surgical disorders with normal AMA. For example, case #42 was that of a 62-year-old woman with severe pelvic pain and a bone mass on CT scan consistent with tumor or hematoma. However, the AMA test was normal. Open biopsy revealed a benign healing fracture. Case #43 was that of a 36-year-old man with a high frequency of cancer in close relatives; severe lum-

bar pain had a new sclerosing lesion fusing two vertebrae. After three elevated AMA tests (Net TAG 229, 407, 197), the resolution of the lesion after a 3-month period was accompanied by a normal AMA (Net TAG 120). Case #44 was that of a 78-year-old man with severe depression and marked weight loss. The AMA test was normal. The depression disappeared, normal weight and health returned. Case #45 was a patient with persistent cancer-phobia. The AMA test was normal. Cases #46 and #47 were elderly patients with severe weight loss suspected of having cancer. After an initial false-positive AMA, the repeat determination was normal, weight was gained, and the patients were felt to be free of cancer and were well 1 year later. The AMA test in sera submitted from 70 normal healthy individuals, cases #48 through 117, all were normal. Note however that case #1, originally one of the "normal healthy control" group, was found to have an elevated AMA test, abnormal gynecological cytology, and cancer of the uterus, which was promptly treated (see also Table II for longitudinal data in monitoring this patient pre- and postoperatively).

For the *Early Detection* group, there were no false negatives. The frequency of false positives on first determination was 4 of 118 (3%). All four had normal AMAS on repeat determination within 2 months, and were well 1 year later. Therefore, the frequency of persistent false positives in cases of certain diagnosis was 0 of 118 (0%).

The diagnosis is still uncertain, with occult cancer to be ruled out, in seven patients (#119 through 125), all of whom have had two to five repeated elevated AMA tests over a period of 1 year. Five of these cases are symptomatic; three have a high frequency of cancer in the family. One of these patients, case #119, had been thought for 20 years to have a hiatus hernia until repeated AMA tests prompted a renewed search for other pathology. Gastroscopy showed erosive gastritis and a small prepyloric ulcer, biopsies of which showed only inflamed and fibrotic mucosa. The patient was treated symptomatically. Follow-up gastroscopies 6 and 14 months after the first AMA test showed no evidence of ulcer and resolution of erosive gastritis. Relief of symptoms was associated with a drop in AMA level, however, not to normal levels. Case #120 is that of a 32-year-old woman with thyroiditis who has a family history of 12 blood relatives with cancer. Cases #121 and #122 also have a high frequency of family cancer. (See also "Added in Proof No. 2.")

Section B in the Table I, *Monitoring*, and Table II, illustrate that in 68 determinations in 56 known cancer patients under treatment, the AMA level, elevated or normal, agrees with the clinical status in a wide variety of malignancies. The diagnosis was confirmed by histopathology in all monitoring cases. Elevated AMA indicated the presence of clinically active nonterminal disease in 36 of 40 determinations (90%), regardless of the type of cancer, the staging, the presence of metastases, and the type of treatment received. In 4 of 40 determinations (10%), indicated in the table by *"D"* (disagreement), elevated AMA levels were not accompanied by clinical evidence of the presence of residual disease, although one of these cases (#146) has had only a 3-month follow-up. Normal values of AMA correlated with "no clinical evidence of residual disease" in 15 of 15 cases (100%). Normal values also correlated with the terminal state in 8 of 8 cases (100%), that is, all died within 1 year. In contrast, of the patients with elevated AMA, only 6 of 38

TABLE II
Early Detection and Monitoring of Cancer of the Cervix in a 35-Year-Old Patient with Anti-Malignin Antibody in Serum

Days before (–) and after (+) surgery	AMA (μg/ml serum)
–24	130 (Borderline)
–1	377 (Elevated)
+47	310 (Elevated)
+90	230 (Elevated)
+97	65 (Normal)
+104	82 (Normal)
+133	85 (Normal)
+160	75 (Normal)
+187	93 (Normal)

(15.8%) died within 1 year (2 have not yet been followed for 1 year). The overall accuracy of the predictive correlation of the AMA level with the clinical status in the *Monitoring* group was 64 of 68 determinations correct (94.1%).

IV. DISCUSSION

A. Validity and Efficacy

Although the number of cases tested by each participating physician, with a few exceptions, was relatively small, the pooled data are of interest. That the types of cancer represented are typical of the cross section that might be encountered in typical practice supports the randomness of distribution of the sample.

The fact that all major forms of cancer, regardless of tissue type, are detected by the AMA test[7-9] is here independently confirmed.

1. Early Detection

In the *Early Detection* group, where the clinical diagnosis was later certain, the efficacy of the test in distinguishing benign from malignant states was 118 of 118 (100%). In the seven remaining cases, all with repeat positives, the diagnosis is still uncertain. If all 7 patients do not have cancer, the maximum possible persistent false-positive rate would be 7 of 125 (5.6%), although considering the accuracy achieved in the 118 cases where the diagnosis was certain, it is likely that in some of these 7 the diagnosis will be occult cancer (see below).

2. Monitoring

It is worth emphasizing that the correlation of a biological variable like AMA with clinical cancer is dependent on as careful as possible a definition of the clinical status and the stage of the disease. A patient who is reasonably well but with active clinical cancer is very different clinically from one depleted by the disease in its advanced or terminal stages, and each of these is different from one who has been in remission without clinical evidence of disease for 1 to 15 years. The immunological integrity, as represented by the AMA level, has been reported to reflect or anticipate these clinical states.[7-9] In the present results, the overall correlation between level of AMA and clinical status was 94.1% in the *Monitoring* group, 100% in the *Detection* group when clinical diagnosis was certain, and 97.9% in both groups combined.

The correlation of normal AMA values with cancer in *Remission* (100%) agrees with earlier studies, which demonstrated that 94.2%[7] and 96.9%[8] of two different groups of successfully treated cancer patients with no clinical evidence of residual disease had normal AMA levels.

In patients with clinically active nonterminal disease, the correlation with elevated AMA is 100% in the *Early Detection* group, and 90% in the *Monitoring* group, regardless of the tissue type of cancer, the staging, the presence of metastases, and the type of treatment received, also confirming earlier data.[7-9] For the 4 of 61 (21 under A + 40 under B in Table I = 61) instances of disagreement between elevated AMA and the clinical impression (D in the table), other than laboratory error, it is possible that the elevated levels of AMA reflect the continued presence of active cancer cells perhaps in numbers too small to produce clinical signs or symptoms. Relevant to this point, case #168 demonstrates that 4.5 months after hepatic lobectomy for metastases from cancer of the colon (Duke's B2, removed 6 years earlier) the AMA was elevated, but that by 6 months post-lobectomy, the AMA was normal; 11 months post-lobectomy the CT scan was negative and there was no clinical evidence of disease, and 18 months post-lobectomy, the patient clinically still shows no evidence of disease. It is possible that the 5- to 6-month period following surgery during which time AMA was elevated (3 months as in case #1, Table II is more usual[7,8]) represents the time required for decay in AMA levels in the patient free of cancer. Furthermore, the change from an elevated to a normal AMA value, which preceded the clinical evidence of no disease in a well patient by almost 5 months in this case, should be looked for in other cases as an

early signal of remission. It would be useful to have data on the half-life of the existing antibody in these cases.

3. Relationship of AMA Level to Survival

Only 15.8% of the cancer patients with elevated AMA and clinically active disease in the *Monitoring* group, and 21% in the *Early Detection* group, died with 1 year (two not yet followed 1 year). In contrast, of those with normal AMA and clinically active disease, 100% were dead in 1 year (*Terminal Cancer*). These findings are in agreement with the earlier observations in and actuarial survival analysis of 511 cancer patients,[7,8] which showed that the level of AMA in cancer is quantitatively related to survival and decreases to "normal" levels in *Terminal Cancer*. Longitudinal studies of individual patients show that a drop in AMA from elevated levels (>134 μg/ml) to normal levels signals death within a few months.[7]

Although distinguishing between the two states of *Terminal* and *Remission*, inasmuch as both have normal AMA values, might appear to be a potential problem, in our experience in routine practice the clinical status of the patient clearly distinguishes between these two states, as it did in previous observations.[7,8]

B. Indications for the Test

The overall frequency of cancer detected in the *Early Detection* group for patients with a confirmed diagnosis, 21 of 125 (16.8%) or 21 of 55 (38.2% if the healthy controls are not included) is obviously greater than the frequency in the general population. This high yield reflects the fact that the physician ordered the test under two indications: (1) when signs and symptoms suggested cancer in the differential diagnosis, or (2) when the patient requested the test. From the yield in terms of cancer detected with each indication, 37.5 and 20%, respectively, these are both effective indications for performing the test. Although the highest positive yield is expected to be favored by testing older symptomatic patients, 6 of the 21 cancer cases found (28.6%) were asymptomatic and 4 (19.1%) were under 60 (mean age 46).

C. Occult Cancer and Immunosurveillance

There is growing evidence of the prevalence of occult cancer. This is illustrated by the high frequency of prostate cancer discovered at autopsy.[11] In our data, there is only the assumption of occult cancer, for example, in cases #43 and #119 where the AMA was at first elevated repeatedly, then with clinical evidence of resolution of a lesion AMA returned toward or to normal values, and cases #120 to 125 with persistent repeated elevated AMA. To prove this assumption would require longitudinal histopathological evidence in individual patients' malignant cells, and their subsequent arrested growth or disappearance, correlated with AMA level or some other immunological process. The availability of the AMA test, perhaps in the future together with the use of radiolabeled human AMA to pinpoint the site *in vivo*,[6] to detect these transformed cells could offer new opportunities in preventive medicine for the interruption of these malignancies.

D. Limitations

Technical and sociological variables each need attention to maintain optimal AMA test efficacy and utility. Technically, there is the need to collect, ship, and determine sera exactly as described in Section II with careful quality control. However, the biggest problem, shared with other cancer tests like the cervical cytology and the mammogram, is that cancer produces fear, which can block the patient from going to the physician for regular check-ups and at the earliest symptoms or signs for complete work-up.

While there is reluctance to use diagnostic laboratory procedures in disorders for which effective treatment is not available, as in some genetic disorders, this constraint is not applicable to cancer diagnosis. In cancer, where life-saving treatment is often available when detection is early, there is an imperative to do everything possible to

make early diagnoses. The frequency of problems, and the attendant stress, due to the inability to detect the site of the malignancy, or the time taken for a definitive diagnosis to rule out cancer, can be estimated: (1) in the present group, 7 patients with uncertain diagnosis and the 4 with transient false positives out of the 181 studied (4 and 2%); (2) in screening 503 industrial workers with three blind AMAS determinations each, only 2 had persistent elevated false-positive AMAS levels (0.4%).[12]

V. CONCLUSIONS

These results indicate for the first time that in routine clinical practice the *in vitro* AMA test is a test for cancer, regardless of tissue type, useful at a high level of accuracy for both the detection and management of cancer. The results are in agreement with the 3315 double-blind tests reported earlier.[7-9] It is important to remember that, as in all clinical laboratory tests, this test is not by itself diagnostic of the presence or absence of disease, and its results can be assessed only as an aid to diagnosis, detection, or monitoring of disease in relation to the history, medical signs and symptoms, and the overall condition of the patient. The efficacy of the test for screening the population at large has not been examined; we have used it only as a screen for particular indications. Thus, in addition to its continued usefulness in monitoring known cancer patients, from our combined experience, the AMA test should be used, together with other routine diagnostic procedures, whenever signs and symptoms suggest cancer in the differential diagnosis and the need for early detection.

ADDED IN PROOF

1. In recent independent studies by Thornthwaite JT, Derhagopian R, and Reimer W. (Abstr.: Proc Annu Meet Am Assoc Cancer Res 1990; 31:A1550; and FASEB J 1990; 4(7):A1811) to be published in full, on the AMA test in the differential diagnosis and monitoring of patients with abnormal mammograms, the AMA test was elevated in 96% of instances where early breast cancer was found on subsequent biopsy (as small as 1 mm in size, 88% stage I, only 6% metastases), whereas CEA, CA 19.9, CA 15.3, and CA 125 were elevated in only 0 to 16%.
2. Of 170 healthy control subjects, the AMA test was elevated in 5 (2.9%). Four of these with apparently false-positive results developed cancer 2 weeks to 3 years later. Three were in their 30s and one was 64 years of age. The fifth had ulcerative colitis (Bogoch E, Bogoch S. Antimalignin as early warning. Cancer Detect Prev 1993; 17:229).
3. In 176 patients with breast disorders, benign and malignant, and in 174 normal healthy and medical noncancer disorder patients, AMA was elevated in all early breast cancer patients regardless of whether they were first occurrence (N = 65) or recurrence (N = 16) and whether localized (N = 73) or metastasized (N = 8). Successful treatment resulting in *no clinical evidence of cancer*, whether achieved by surgery, chemotherapy, radiation, other, or a combination thereof, was associated with normal AMA values in 83% within 1 year, and in 96% 2 to 27 years after successful treatment (Bogoch, S, Bogoch, ES. Antimalignin antibody returns to normal on successful treatment of breast cancer. Cancer Detect Prev 1993; 17:276).
4. The AMA test is now being introduced in trials in the U.K. National Health screening program for breast cancer where all (6 million) women between the ages of 50 and 64 are being invited to have a mammogram.

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