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Low plasma coenzyme Q_{10} levels as an independent prognostic factor for melanoma progression

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Background: Abnormally low plasma levels of coenzyme Q_{10} (CoQ_{10}) have been found in patients with cancer of the breast, lung, or pancreas.

Objective: A prospective study of patients with melanoma was conducted to assess the usefulness of CoQ_{10} plasma levels in predicting the risk of metastasis and the duration of the metastasis-free interval.

Methods: Between January 1997 and August 2004, plasma CoQ_{10} levels were measured with high-performance liquid chromatography in 117 consecutive melanoma patients without clinical or instrumental evidence of metastasis according to American Joint Committee on Cancer criteria and in 125 matched volunteers without clinically suspect pigmented lesions. Patients taking CoQ_{10} or cholesterol-lowering medications and those with a diagnosis of diabetes mellitus were excluded from the study. Multiple statistical methods were used to evaluate differences between patients and control subjects and between patients who did (32.5%) and did not (67.5%) develop metastases during follow-up.

Results: CoQ_{10} levels were significantly lower in patients than in control subjects (*t* test: $P < .0001$) and in patients who developed metastases than in the metastasis-free subgroup (*t* test: $P < .0001$). Logistic regression analysis indicated that plasma CoQ_{10} levels were a significant predictor of metastasis ($P = .0013$). The odds ratio for metastatic disease in patients with CoQ_{10} levels that were less than 0.6 mg/L (the low-end value of the range measured in a normal population) was 7.9, and the metastasis-free interval was almost double in patients with CoQ_{10} levels 0.6 mg/L or higher (Kaplan-Meier analysis: $P < .001$).

Limitations: A study with a larger sample, which is currently being recruited, and a longer follow-up will doubtlessly increase the statistical power and enable survival statistics to be obtained.

Conclusions: Analysis of our findings suggests that baseline plasma CoQ_{10} levels are a powerful and independent prognostic factor that can be used to estimate the risk for melanoma progression. (J Am Acad Dermatol 2006;54:234-41.)

Melanoma is one of the most aggressive malignant tumors known, and there is currently no truly effective treatment for patients with disseminated disease. Since 1935, the incidence of melanoma has gradually increased. It currently accounts for 2% to 3% of all malignant tumors and is the cause of 1% of all malignancy-related deaths.[1,2]

An important part of the management of melanoma patients is evaluation of the individual risk for development of metastases.[3-5] This assessment should be carried out at the time of diagnosis and during the follow-up. However, studies of the evaluation of prognostic factors (messenger RNA tyrosinase, S-100 protein, enolase, Melan/MART 1, metallothionine, and numerous others) are still in the planning stages.[6-11]

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The staging system developed by the American Joint Committee on Cancer (AJCC) in 2002 is based on evaluation of primary tumor thickness, presence of ulceration, number of metastases and micrometastases to the lymph nodes and/or other sites, and serum levels of lactate dehydrogenase.[12] Breslow thickness is currently considered the most important factor for predicting the outcome of the disease.[13]

In the late 1950s, two groups of investigators[14,15] identified a new substance present in all cells and involved in mitochondrial electron transport in the cells, coenzyme Q_{10} (CoQ_{10}). Because CoQ_{10} is essential for normal cell respiration and function, any deficiency in its synthesis or availability can potentially disrupt normal cellular function. A situation of this type could lead to abnormal cell-division patterns, which might, in turn, induce an oncogenic response.[16-19]

In studies of the blood levels of CoQ_{10} in cancer patients from the United States and Sweden, lower than normal concentrations were found in patients with cancer of the breast, lung, or pancreas.[18-22] Picardo et al[23] reported abnormally low levels of CoQ_{10} (as well as increased levels of polyunsaturated fatty acids, superoxide dismutase, and vitamin E as well as decreased catalase activities) in melanoma cells and in a percentage of normal melanocytes from melanoma patients.

An imbalance in the antioxidant system can lead to endogenous generation of reactive oxygen species that could disrupt the correct cellular reproduction and might therefore correlate with the malignant transformation of cells and disease progression.[24]

The aim of this prospective study was to investigate baseline plasma levels of CoQ_{10} in melanoma patients as a potential predictor of the duration of the disease-free interval and compare its prognostic value with that of other factors, such as Breslow thickness. Gaining better prognostic information not only better informs the patient, but may help define both treatment options and clinical evaluation for metastasis as well as the response to postsurgical adjuvant therapy.

MATERIAL AND METHODS

From January 1997 to August 2004, 126 consecutive patients with primary cutaneous melanoma were seen at the Dermatology Department of the Catholic University Medical Center in Rome. Of these, 4 refused to participate in the study and 5 did not return after the initial visit, leaving 117 patients. Clinical staging was performed according to AJCC 2002 criteria.[12] The imaging techniques used were ultrasonography, chest radiography, computed tomographic scanning, and magnetic resonance imaging. Patients with melanomas of Breslow thickness of 1 mm or more who were recruited in 2002 underwent sentinel node biopsy (n = 18). The group included 79 patients with stage 1 and 2 disease and 38 with stage 3 and 4 disease (Table I). None had clinical, histologic, or instrumental evidence of regional or distant metastatic disease.

Table I. Features of patients with melanoma

Observation time (mo)	
Mean	34.44
SD	18.36
Median	32
Range	5.90
Age (y)	
Mean	55.44
SD	16.48
Median	56
Range	20-88
Sex	
Male	61
Female	56
Location of primary melanoma	
Thorax	49*
Upper arm	55
Neck	0
Scalp	13
Other site	0
Tumor thickness (mm)	
<1	46*
1.01-2.0	21
2.01-4.0	43
>4.01	7
Tumor stage	
I-II	79*
III-IV	38

*Number of patients.

Patients with histopathologic diagnosis of malignant melanoma were included regardless of clinical stage (from melanoma in situ to AJCC stage IV). Patients taking CoQ_{10} or cholesterol-lowering medications and those with other malignancies or a diagnosis of diabetes mellitus were excluded from the study. As the control group, we studied 125 volunteers from the clinic's population without clinically suspect pigmented lesions matched for sex, categorical age, occupation, and region of birth.

All subjects gave their written informed consent to participate in the study.

Plasma levels of CoQ_{10} were measured in all melanoma patients and control subjects by sing the method of Littarru et al.[25] A cut-off point of 0.6 mg/mL was adopted to divide plasma CoQ_{10} levels into high and low groups based on the normal range (0.6-1.0 mg/mL) measured in a selected normal population similar to our patient population for

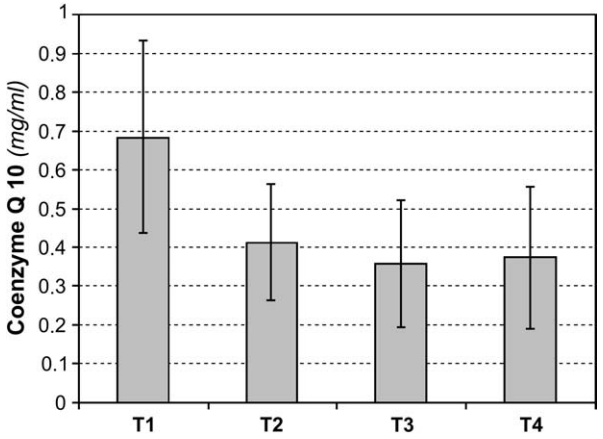

Fig 1. Plasma levels of CoQ_{10} in melanoma patients. Correlation with Breslow thickness.

lifestyle and dietary habits.[26] This range is in agreement with other studies.[27,28]

Coenzyme Q_8 was used as the internal standard. The mean recovery rate was 90% ± 5%. The linearity range was 0.2 to 10 μg/mL. The variation coefficient for total imprecision was 3.6%. Total run time for each sample was 10 minutes. Retention times were 5.2 and 8.3 minutes for CoQ_8 and CoQ_{10}, respectively.

Follow-up (mean, 34.44 months; range, 5-90 months) was performed at 3- or 6-month intervals according to disease stage and patient conditions and included physical examination, laboratory tests, and imaging studies (as appropriate).

Statistical analysis

Data were analyzed with the Statistical Package for the Social Sciences software for Windows (version 9.0, Chicago, Ill). A *t* test for independent samples was used to detect differences in baseline levels of CoQ_{10} between melanoma and control subjects. The melanoma group was also divided into two subgroups based on the presence or absence of metastases, and differences between the CoQ_{10} levels of these two groups were evaluated with the Student *t* test. The same approach was used to evaluate differences related to sex and to the location of the primary tumor. In the melanoma group, correlation between Breslow thickness and CoQ_{10} levels was evaluated by means of the Pearson correlation coefficient. Analysis of variance was used to identify differences in CoQ_{10} levels between patient subgroups defined by TNM stage (T1, T2, T3, T4). To determine whether plasma levels of CoQ_{10} could be used to predict metastasis, we constructed a logistic (regression) model with "development of metastases" as the dependent variable (two levels: 0 and 1) and "CoQ_{10} level" (two levels: low or high) as the independent variable; we estimated the odds ratios. Kaplan-Meier curves and the log rank test were used to evaluate disease-progression times in melanoma patients with low (<0.60 mg/L) versus high (≥0.60 mg/L) levels of CoQ_{10}. Cox regression analysis was used to investigate progression times, by using CoQ_{10} levels (high or low), TNM stage, and patient age as potential prognostic factors. In all analyses, an α level of 5% was considered significant.

RESULTS

The melanoma patients studied were 56 women and 61 men with a mean age of 55.44 ± 16.48 years (range, 20-88 years). The melanoma was located on one of the limbs in 55 patients, on the trunk in 49, and on the head in the remaining 13.

The mean plasma level of CoQ_{10} was significantly higher in the control group than that in the melanoma group (1.271 ± 0.614 mg/L vs 0.497 ± 0.251 mg/L; $P < .0001$). We measured differences in CoQ_{10} plasma levels between patients with (n = 38) and without (n = 79) metastases. Interestingly, in the subgroup that did develop metastases, mean baseline values (0.342 /l ± 0.17 mg/L) were significantly lower ($P < .0001$) than those found in the patients who did not go on to develop metastases (0.572 ± 0.248 mg/L).

No significant differences were found between CoQ_{10} levels in male compared with female patients or in subgroups defined by the site of the tumor (photoexposed vs nonexposed skin). In contrast, there was a significant ($P = .024$) positive (0.208) correlation between CoQ_{10} levels and patient age, with patients older than 60 years exhibiting higher values than younger patients.

Plasma CoQ_{10} concentrations displayed a significant negative correlation (−0.544) with Breslow thickness ($P < .0001$). Furthermore, analysis of variance revealed significantly different levels of CoQ_{10} in patient groups defined by the TNM stage ($P < .0001$). In particular, the mean level of T1 patients (0.68 ± 0.25 mg/L) was significantly higher ($P < .001$) than those found in the other subgroups (T2 = 0.41 ± 0.15 mg/L; T3 = 0.38 ± 0.17 mg/L; T4 = 0.32 ± 0.15 mg/L) (Fig 1).

The results of logistic regression analysis indicated that plasma levels of CoQ_{10} are a significant predictor of metastasis ($P = .0013$). Patients with low CoQ_{10} levels had an approximate 8-fold risk of metastatic disease than patients with higher levels (95% confidence interval, 2.2491-28.0509) (Table II).

The time to metastasis for melanoma patients with low and high levels of CoQ_{10} is shown in Fig 2. Of the 35 patients with higher than normal levels,

Table II. Occurrence of metastases during follow-up in melanoma patients with high and low baseline levels of plasma CoQ_{10}

	CoQ_{10} plasma level (mg/L)			Odds	Odds ratio	*P*
	≥ 0.6	<0.6	Total	ratio	CI	value
Patients without metastasis	32	47	79			
Patients with metastasis	3	35	38	7.94	2.25-28.05	.0013
Total	35	82	117			

CI, Confidence interval.

3 (8.9%) developed metastases during the study (mean time, 81.53 ± 4.62 months from diagnosis), compared with 35 of the 82 patients (42.7%) with lower than normal concentrations (mean time, 46.85 ± 3.49 months from diagnosis).

The log rank test revealed that the distributions of progression times were significantly different ($P < .0001$) in these two subgroups (Table III).

Age, Breslow thickness, and CoQ_{10} plasma levels were analyzed by means of a Cox regression analysis. The only significant prognostic factor was Breslow thickness, which was recoded in the analysis according to the T classification. In particular, when CoQ_{10} was inserted in the regression analysis along with T, its regression coefficient was not significant. This may be due to the existence of a correlation among CoQ_{10} and T. However, since the significance was borderline ($P = .0728$) and the backward stepwise criteria (Wald statistic) were satisfied, CoQ_{10} was still retained in the final model. The odds ratios for patients classified as T3 or T4 were, respectively, 6.77 (95% confidence interval [CI], 2.0942-21.8882) and 8.2298 (95% CI, 2.3690-28.5904). The overall chi-square statistic was highly significant ($P < .001$) (Table IV).

To determine whether the probability of a metastasis developing in these patients depends on Breslow thickness, logistic regression analyses were performed, first considering the thickness as a continuous variable and then after recoding the variable according to the T classification. The former analysis revealed that this probability increased with increasing Breslow thickness ($P < .001$). Specifically, odds ratios were computed for T2, T3, and T4 with respect to the baseline level, T0. Patients in classes T2 and T3 had a significantly higher risk of metastasis (T2: 13.4996, $P < .001$; T3: 20.9993, $P < .001$) than those with Breslow thickness of less than 1. Results are reported in Table V.

Fisher's exact test revealed a significant association between death and levels of plasma CoQ_{10} ($P = .003$). Table VI shows the frequency distribution of melanoma patients according to CoQ_{10} concentrations and survival.

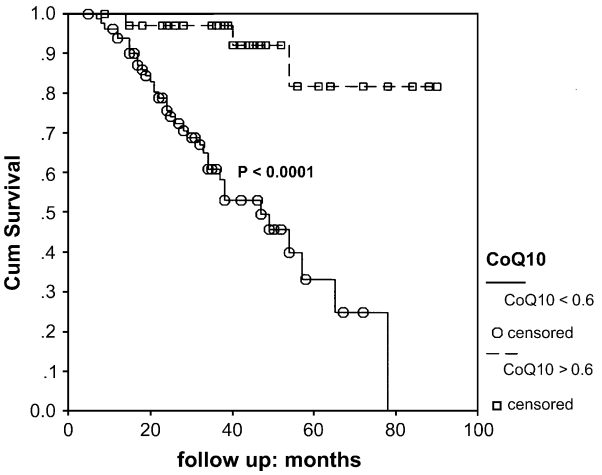

Fig 2. Time to metastasis for melanoma patients with low and high concentrations of CoQ_{10}.

DISCUSSION

In recent years, there has been a considerable increase in the frequency of melanomas.[1,2] One of the most frightening characteristics of these tumors is their ability to produce distant metastases years after diagnosis and surgical removal of the primary tumor. The adjuvant postoperative therapies used to date have produced results that for the most part are discouraging, especially in patients with stage III or IV disease.[29]

Estimating the risk of progression in individual patients, preferably at the time of primary diagnosis, is very important. To date, Breslow thickness is considered to be the best predictive factor even though the tumor progresses in 5% to 22% of patients with thin lesions (<1.5 mm), whereas in some patients with thick melanomas, metastases never develop during their lifetime.[30]

Various groups have stressed the need for markers that can be used for reliable staging of melanomas and for predicting the risk for disease progression and the potential response to adjunctive postoperative therapies. No definitive conclusions can be drawn on the relative merits, in terms of cost, feasibility, reproducibility, and, above all, reliability, of the potential prognostic variables investigated to date (eg, messenger RNA tyrosinase, the S-100 protein, enolase, Melan/MART 1, metallothionine).[6-13,31]

Statistical analysis of the data collected in our study indicates that baseline plasma CoQ_{10} levels are a powerful and significant independent prognostic

Table III. Time to metastasis for melanoma patients with low and high levels of CoQ_{10}: Results of Kaplan-Meier analysis

CoQ_{10} plasma level (mg/L)	Total (No. of patients)	No. of events	No. censored	% Censored	Mean %	SE	CI (Lb-Ub)	Log rank test (*P* value)
≥0.6	35	3	91.43	91.43	81.53	4.62	72.48-90.58	
<0.6	82	35	47	57.32	46.85	3.49	40.00-53.70	<.001

Lb-Ub, Lower bound and upper bound of the confidence interval of logistic regression analysis.

Table IV. Analysis of patients' age, baseline CoQ_{10} concentration, and T classification as predictors of time to metastasis

Variable	B coefficient	OR	*P* value	CI for exp(B) (Lb-Ub)
Age	0.0039	1.0039	.7128	0.9835-1.0247
CoQ_{10}	1.1944	3.3016	.0728	0.8953-12.1757
T1				
T2	0.3117	1.3657	.6680	0.3287-5.6748
T3	1.9126	6.7704	.0014	2.0942-21.8882
T4	2.1078	8.2298	.0009	2.3690-28.5904

CI, Confidence interval for regression coefficient B; *exp(B),* exponential of the regression coefficient B; *Lb-Ub,* lower bound and upper bound of the confidence interval of logistic regression analysis; *OR,* odds ratio.

Table V. Results of logistic regression analysis to determine whether the probability of metastasis in melanoma patients depends on Breslow thickness (according to T classification)

T classification (Breslow thickness)	OR	*P* value	OR CI (Lb-Ub)	
T1				
T2	2.47	.24	0.55-11.03	0.55-11.03
T3	13.50	<.001	3.90-46.69	3.90-46.69
T4	21.00	<.001	5.08-86.75	5.08-86.75

CI, Confidence interval; *Lb-Ub,* lower bound and upper bound of the confidence interval of logistic regression analysis; *OR,* odds ratio.

factor that can be used to estimate the risk of disease progression in melanoma patients. Its prognostic value was demonstrated by means of various types of analysis, including *t* test, Pearson correlation coefficient, Kaplan-Meier curves, and Cox univariate and multivariate analyses. In particular, we found that, compared with melanoma patients, subjects without melanoma displayed significantly higher mean levels of plasma CoQ_{10}.

On the basis of these preliminary observations, we could begin to regard this substance as a new tumor marker and to acquire further information on the carcinogenesis of melanoma. One of the main functions of CoQ_{10} is its antioxidant activity, which protects cells against damage by free radicals. CoQ_{10} is thought to stabilize cell membranes (lipid-containing structures essential to maintaining cell integrity) and to prevent free radical damage to other important cellular components. Free radical damage to DNA (and possibly to other cellular molecules) may be a factor in cancer development.[16-19]

A previous study has shown that the redox status of a cell can influence the transcription of nuclear factor−kB, which is important for the neoplastic transformation of melanomas and other types of tumor.[24] In melanomatous cell cultures, this factor appears to be constitutionally activated by the spontaneous production of superoxide anions and by overexpression of NAD(P)H:quinone oxidoreductase. By reducing membrane ubiquinone levels, this could lead to formation of dioxide through an autocrine mechanism.[24] In vitro studies have demonstrated that antioxidants can exert antiproliferative effects based on their ability to reduce nuclear factor−kB activation.[21,32]

Studies conducted on lymphocytes and melanoma cells have demonstrated an imbalance in the antioxidant pool in tumor cells compared with normal cells and a reduction in plasma levels of CoQ_{10} in patients with cancer. This finding strengthens the hypothesis that some subjects are genetically predisposed to oxidative damage.[18,23,24,33-37]

A role for CoQ_{10} in cancer was first surmised in 1961, when a deficiency was noted in the blood of Swedish and American patients with cancer, especially of the breast.[18] A subsequent study showed a significant correlation between the level of plasma CoQ_{10} deficiency and prognosis of breast cancer.[38] Low blood levels of this compound have also been reported in patients with other malignancies, including myeloma, lymphoma, and cancers of the lung, prostate, pancreas, colon, kidney, head, and neck.[18,39] Furthermore, decreased levels of CoQ_{10} have been detected in malignant human tissue.[40,41] A large body of laboratory and animal data has since accumulated showing that CoQ_{10} stimulates the immune system, leading to higher antibody levels, greater numbers and/or activities of macrophages and T cells, and increased resistance to infection. CoQ_{10} has also been reported to increase IgG levels

and the CD4/CD8 T-cell ratio, which decreases in cancer patients.[33-37]

CoQ_{10} is essential to aerobic energy production, and it has been suggested that increased cellular energy leads to increased antibody synthesis in B cells (B lymphocytes).[33-37] Cellular energy production, stimulation of the immune system (which may be related), and the antioxidant properties of CoQ_{10} are all relevant to cancer.[16-19,35,36]

Our data showed significantly lower mean baseline plasma levels of CoQ_{10} in patients in whom the tumor progressed than in those who did not go on to develop metastases. Numerous studies have demonstrated the importance of an adequate immune host response in hindering the metastatic diffusion of melanoma cells and its relation to the tendency of tumor clones to express surface antigens, particularly those belonging to major histocompatibility class I.[42] Differentiation of these cells, following molecular alterations in the antigens expressed on their surfaces, can give rise to cellular clones that are no longer recognized by the immune system, with highly malignant phenotypes and a marked capacity for metastasis.[42-44]

One of the most successful forms of adjunctive postsurgical therapy is based on the use of immune modulators, which are capable of combating occult micrometastases that may be present after surgical excision of the primary tumor.[45] The rationale is to intervene early to eliminate possible micrometastases that are still undetectable, represented by cells with lower mutagenic activity that are still in a phase in which they cannot completely escape control by the immune system.[45] CoQ_{10} plays a key role in mitochondrial adenosine triphosphate (ATP) production, which is indispensable for antibody production.[33-37] One might therefore speculate that melanoma patients, especially those with metastatic disease, could be characterized not only by an imbalance in the antioxidant pool, but also by an increased demand for ATP because of the increased immune response, which could result in an increase in active transport of CoQ_{10} from plasma to cells.[16-22,37]

Although late metastasis has been documented in 5% to 22% of patients with "thin melanomas," Breslow thickness is still considered the most reliable predictor of disease progression.[30,46] In the patients we examined, those with a T1 classification, whose tumors were less than 1 mm thick, had significantly higher levels of CoQ_{10} than those with T2, T3, or T4 tumors.

Patients with CoQ_{10} concentrations lower than the normal range (0.60-1.00 mg/L) had an 8-fold risk of disease progression than patients with higher-than-normal levels. CoQ_{10} levels did not vary significantly with the site of the primary tumor (photoexposed or nonexposed skin) or the sex of the patient. Higher levels were observed in patients 55 to 60 years of age and older, probably because of the decreased demand for ATP caused by age-related slowing of metabolic activities. This finding might explain differences in the biological behavior of melanomas in this age group. Other studies have shown that older patients with melanoma have a lower risk for lymph node and local-regional metastases than their younger counterparts.[31] Plasma levels of CoQ_{10} also emerged as a highly significant predictor of the duration of the disease-free interval, as the time to recurrence was significantly longer in patients with higher levels.

Table VI. Association between survival of melanoma patients and their levels of plasma CoQ_{10}

	CoQ_{10} plasma level (mg/L)			Fisher's exact test (two-sided)
	≥0.6	<0.6	Total	
Living (No. of patients)	35	65	100	
Deceased	0	17	17	0.003

Identification of patients with cutaneous melanoma with low risk as opposed to high risk of metastasis is an important goal that will improve our ability to optimize treatment for these tumors. The preliminary results obtained in this first study of CoQ_{10} levels in patients with melanoma have encouraged us to continue and expand our investigation. Work is currently under way to address its main limitations; the patient sample will be enlarged to increase statistical power and to study a more varied geographic population, while a longer follow-up will allow to obtain survival statistics.

CONCLUSIONS

Findings that have emerged from this preliminary study indicate the following:

- CoQ_{10} concentrations in melanoma patients were significantly lower than those of control subjects.
- These levels had a significant correlation with the thickness of the primary tumor, with the highest levels being observed in patients with the thinner tumors.
- Patients in whom metastases developed had lower levels of CoQ_{10} than those who did not.
- The disease-free interval was shorter in patients with lower baseline levels of CoQ_{10}.
- Although Breslow depth remains a more powerful predictor of melanoma outcome than CoQ_{10} levels, according to our regression analysis it fails to accurately predict outcome in many patients,

leaving a considerable part of the prognostic variation unexplained. Efforts under way to develop effective adjuvant therapies for patients with high-risk malignant melanoma further highlight the need for reliable methods to select patients with a high risk of relapse and for techniques to monitor response to therapy and detect relapse early.

On the whole, our experience suggests that reduced plasma concentrations of CoQ_{10} are a probable tumor marker as well as a significant predictor of future metastases in patients with melanoma. Evaluation of CoQ_{10} levels could thus be a useful addition to melanoma follow-up protocols. Assays of plasma levels of CoQ_{10} is easily performed in laboratories that routinely carry out biochemical analyses, the results can be obtained rapidly, and their cost is limited.

Further studies are therefore needed before determination of CoQ_{10} levels in serum can be used in routine clinical practice to supplement, or perhaps to some extent replace, conventional methods for measuring progression and regression of the disease.

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