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# Presence of Poorly Stained Myocytes in Acute Myocarditis Predicts Improvement in Cardiac Function

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## Abstract

Histological findings in the acute phase of myocarditis were evaluated as a prediction of hemodynamic state in the chronic phase in 20 patients with clinical and pathological diagnoses of myocarditis who were followed up with echocardiography for at least 1 year. Endomyocardial biopsy samples were obtained from the left ventricle within 1 year of the onset of symptoms. Azan-Mallory staining was performed on the myocytes, which were categorized as either well stained or poorly stained. The point counting method was used to determine the fraction of each type.

The improvement in ejection fraction within 1 year correlated significantly with the fraction of poorly stained myocytes ($r=0.46$, $p<0.05$). The ejection fraction at biopsy was negatively correlated with the volume fraction of well stained myocytes ($r=-0.64$, $p<0.01$). The staining condition of myocytes may be useful in predicting the hemodynamic recovery of patients with myocarditis.

**Key Words**
**Myocarditis, Pathology, Prognosis, Ventricular function, Endomyocardial biopsy**

## INTRODUCTION

Myocarditis exhibits a clinical spectrum ranging from normal cardiac function to chronic congestive heart failure indistinguishable from idiopathic dilated cardiomyopathy[1,2]. Cardiac function deteriorates greatly in some patients in the acute phase of myocarditis and may return to normal in the chronic phase; in others the reverse is true[3]. Endomyocardial biopsy is increasingly performed in patients with myocarditis to obtain diagnostic and prognostic information[4–6]. No correlation was found between the volume fraction of collagen tissue and hemodynamic parameters[5]. The histologic features of myocarditis, such as inflammatory cell infiltration, myocardial hypertrophy, interstitial fibrosis and myocardial degeneration, did not influence patient survival[6]. However, whether the morphologi-

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cal features of myocarditis influence the long-term prognosis or the state of cardiac function is unclear.

This study attempted to clarify whether hemodynamic recovery in the chronic phase of myocarditis can be predicted from endomyocardial biopsy findings in the early stage of the disease.

**Selected abbreviations and acronyms**

EF=ejection fraction
NYHA=New York Heart Association

## SUBJECTS AND METHODS

### Study population

There were 20 patients, 11 men and 9 women, aged 29 to 71 years (mean age 55±12 years), with clinical and pathological diagnoses of myocarditis. The clinical diagnostic criteria for myocarditis[4-10] were congestive heart failure developing after influenza-like symptoms with chest pain or discomfort, unexplained congestive heart failure in the absence of severe coronary artery or valvular heart disease, ventricular failure disproportional to demonstrable valvular or coronary heart disease, electrocardiographic abnormalities and an associated increase in serum cardiac enzymes, and positive findings of neutralizing viral antibody in serum. The pathological criteria, modified from previous studies[2,7-10], were inflammatory cell infiltration with monocytes, and preservation of nuclei for cell necrosis in myocardial fibers adjacent to the inflammatory cell infiltration. No patients received immunosuppressive therapy.

### Catheterization

All patients underwent left ventricular catheterization including selective coronary arteriography and left ventricular cineangiography 3.6±4.2 months after the onset of myocarditis. Following catheterization, endomyocardial biopsy was performed with an Olympus bioptome via the retrograde transarterial route. Three or four biopsy samples were taken from the left ventricular posterolateral free wall in all patients.

### Point counting method

Biopsy specimens fixed in 10% formalin were dehydrated with a series of ethanol solutions and embedded in paraffin. Thin sections were prepared from the paraffin blocks and examined under a light microscope. Each biopsy specimen was stained with Azan-Mallory agent and examined microscopically (magnification ×40) on a grid to differentiate the well and poorly stained myocytes. Cross points falling on the well and poorly stained myocytes, except endocardial fibrosis, were analyzed to determine the volume fraction for each histological section (volume fraction=total count divide by number of cross points)[11-14]. Two pathologists without knowledge of the clinical data measured each volume fraction independently (**Figs. 1-A, B**). A third determination was made when the interobserver difference in each count exceeded 5%. The interobserver variability of the volume fraction for each finding was small (4.5±2.0%, $n=6$). The error in the repeated measurements (intraobserver variation) was also less than 5%. The total count was 1,677±755 in each patient.

### Hemodynamic data

Cardiac function study was followed by echocardiography. Left ventricular ejection fraction (EF) was calculated using Gibson's formula[15], and the improvement was evaluated as the maximal change (ΔEF) in ejection fraction within 12 months of biopsy.

### Statistical analysis

Data were reported as mean±standard deviation. Wilcoxon's $t$-test for unpaired data and linear regression analysis were used. A level of $p<0.05$ was accepted as statistically significant.

## RESULTS

### Clinical data

No patients died during the 12-month follow-up period (**Table 1**). Ejection fraction was 0.43±0.10 at biopsy and 0.54±0.12 at the follow-up. Left ventricular end-diastolic and end-systolic dimensions at biopsy were 63.2±7.3 mm and 51.6±8.5 mm, and thereafter decreased at follow-up to 58.7±8.6 mm and 44.7±10.0 mm, respectively. Twelve patients showed an increase in ejection fraction of 0.10 or more (group 1), and the remaining eight patients had an increase in ejection fraction of less than 0.10 (group 2), including two patients whose condition had deteriorated.

Volume fractions of the well and poorly stained

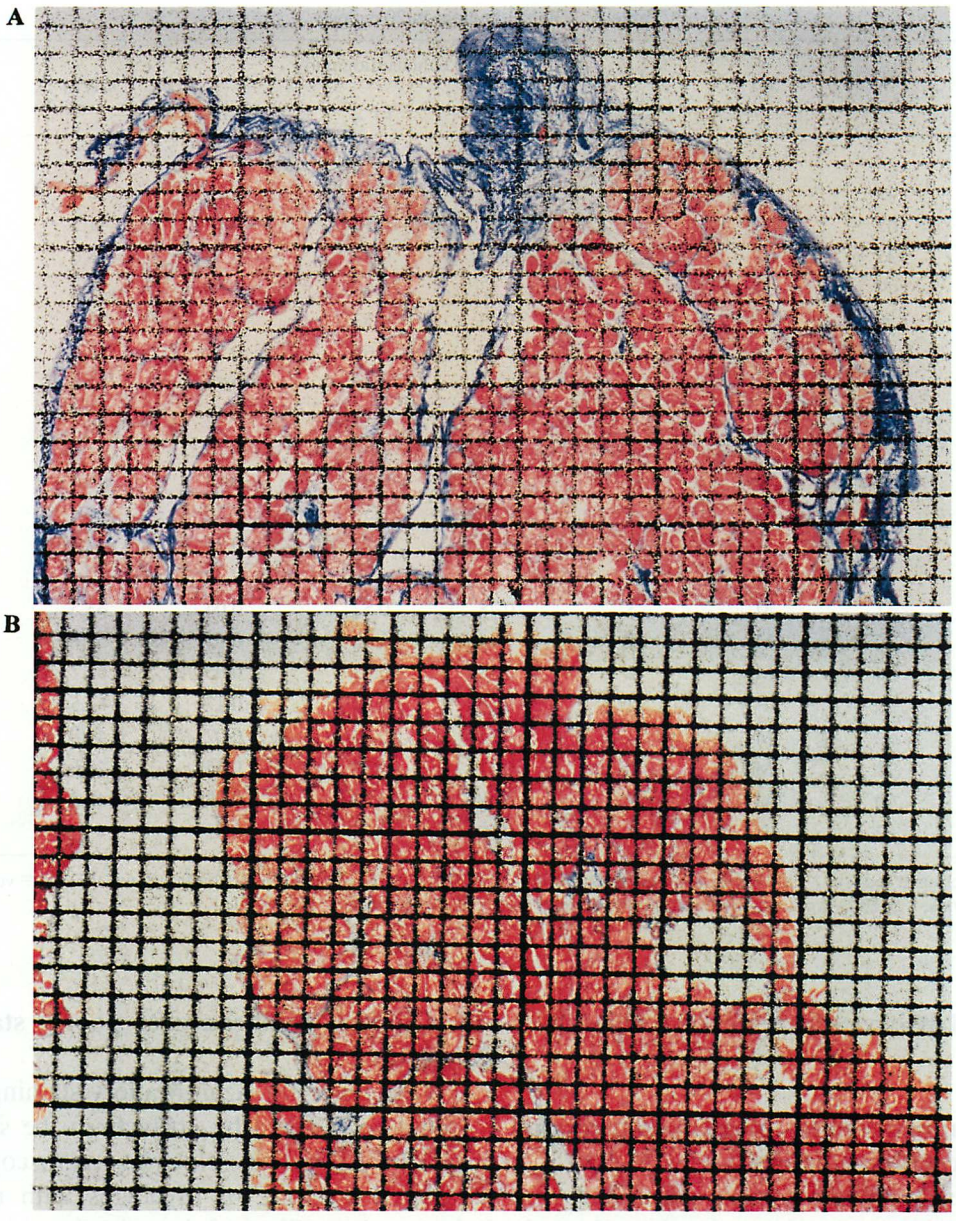


**Fig. 1** Two representative cases of myocarditis

**A** : Case 8. Photograph showing a high volume function of poorly stained myocytes. This patient showed improvement of EF ($\Delta EF = 0.15$).

**B** : Case 15. Photograph showing a high volume function of well stained myocytes. This patient's EF remained unchanged ($\Delta EF = 0.05$).

myocytes in group 1 were $30.5 \pm 16.6\%$ and $32.9 \pm 16.0\%$, while those in group 2 were $50.4 \pm 9.8\%$ and $19.4 \pm 12.3\%$, respectively. Volume fraction of the poorly stained myocytes in group 1, who had a good hemodynamic prognosis, was significantly higher than that in group 2, whose hemodynamic prognosis was relatively poor. There were no significant differences between the groups in age, sex, New York Heart Association (NYHA) functional class, or in the cardiac function at the time of biopsy.

### Correlation between histological features and cardiac function at biopsy

The volume fraction of well stained myocytes was significantly correlated with ejection fraction at biopsy ($r = 0.64$, $p < 0.01$), whereas no significant correlation was found between volume fraction of

**Table 1** Clinical findings in 20 patients

| Case No. | Age (yr) | Sex | NYHA class | Echocardiography | | | %WM | %PM |
|---|---|---|---|---|---|---|---|---|
| | | | | EF 1 | EF 2 | ΔEF | | |
| Group 1 | | | | | | | | |
| 1 | 41 | M | 3 | 0.35 | 0.49 | 0.14 | 42.3 | 23.3 |
| 2 | 65 | F | 2 | 0.44 | 0.56 | 0.12 | 41.3 | 26.0 |
| 3 | 37 | M | 2 | 0.31 | 0.51 | 0.20 | 38.0 | 17.6 |
| 4 | 49 | M | 2 | 0.53 | 0.66 | 0.13 | 39.6 | 28.0 |
| 5 | 58 | F | 3–4 | 0.43 | 0.53 | 0.10 | 38.6 | 16.4 |
| 6 | 56 | F | 2 | 0.48 | 0.69 | 0.21 | 36.1 | 24.8 |
| 7 | 47 | M | 3 | 0.40 | 0.56 | 0.16 | 19.8 | 55.0 |
| 8 | 67 | F | 4 | 0.26 | 0.41 | 0.15 | 2.3 | 60.8 |
| 9 | 53 | F | 2–3 | 0.38 | 0.67 | 0.29 | 7.2 | 47.1 |
| 10 | 45 | M | 3 | 0.51 | 0.69 | 0.18 | 48.3 | 32.0 |
| 11 | 59 | F | 3 | 0.30 | 0.50 | 0.20 | 11.9 | 16.7 |
| 12 | 76 | F | 3 | 0.51 | 0.75 | 0.24 | 27.2 | 50.5 |
| Group 2 | | | | | | | | |
| 13 | 71 | M | 3 | 0.48 | 0.52 | 0.04 | 43.5 | 14.2 |
| 14 | 29 | M | 2 | 0.44 | 0.42 | −0.02 | 59.8 | 8.8 |
| 15 | 50 | M | 2 | 0.58 | 0.63 | 0.05 | 68.7 | 15.1 |
| 16 | 60 | F | 2 | 0.56 | 0.39 | −0.17 | 54.5 | 15.9 |
| 17 | 52 | M | 2–3 | 0.32 | 0.34 | 0.02 | 44.0 | 13.6 |
| 18 | 71 | M | 2 | 0.31 | 0.37 | 0.06 | 40.6 | 9.6 |
| 19 | 60 | M | 2 | 0.48 | 0.51 | 0.03 | 48.8 | 37.2 |
| 20 | 62 | F | 2 | 0.50 | 0.52 | 0.02 | 43.3 | 40.6 |

EF 1=ejection fraction at biopsy; EF 2=ejection fraction at follow-up; ΔEF=improvement of ejection fraction; %WM=volume fraction of well stained myocytes; %PM=volume fraction of poorly stained myocytes; M=male; F=female.

poorly stained myocytes and ejection fraction at biopsy (**Fig. 2**).

### Correlation between histological features and improved cardiac function

There was a significant positive correlation ($r=0.46$, $p<0.05$) between volume fraction of poorly stained myocytes and improved cardiac function (ΔEF). A close negative correlation ($r=-0.64$, $p<0.01$) was observed between volume fraction of well stained myocytes and ΔEF (**Fig. 3**).

## DISCUSSION

### Correlations between volume fraction of well or poorly stained myocytes and ejection fraction improvement

The results suggest that the patients with a higher number of poorly stained myocytes may have a lower tendency toward improvement in cardiac function in myocarditis.

### Clinical significance of poorly stained myocytes

Differences in Azan-Mallory staining conditions could be induced by artifacts in the staining process[16,17]. However, electron microscopic findings showed swelling of myocytes with myofilament loss and swelling of the mitochondria with focal loss of cristae in the poorly stained, but not in the well stained, myocytes (**Fig. 4**). This suggests that the poorly stained myocytes were not artifacts, but may have been caused by water content in colloid of cytozol. This supports our inference that differences in staining condition may reflect the severity of injury in myocytes.

### Correlation between well stained myocytes and cardiac function

Poorly stained myocytes may produce depression of cardiac function, whereas well stained myocytes may preserve cardiac function during the acute phase of myocarditis. We found a close positive

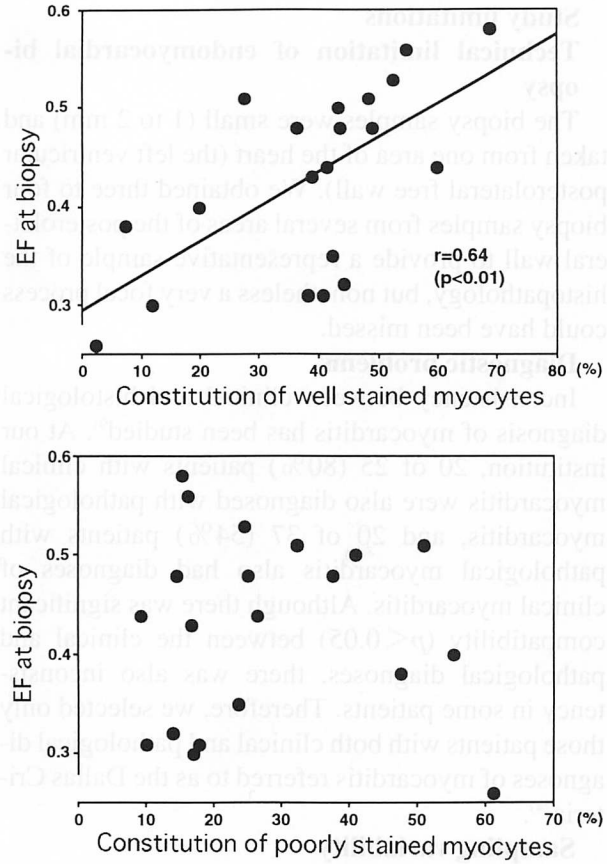


**Fig. 2** Correlation between EF and volume fraction of well stained myocytes (*upper*) and poorly stained myocytes (*lower*) at biopsy

A close correlation was found between the volume fraction of well stained myocytes and EF at biopsy ($r=0.64$, $p<0.01$).

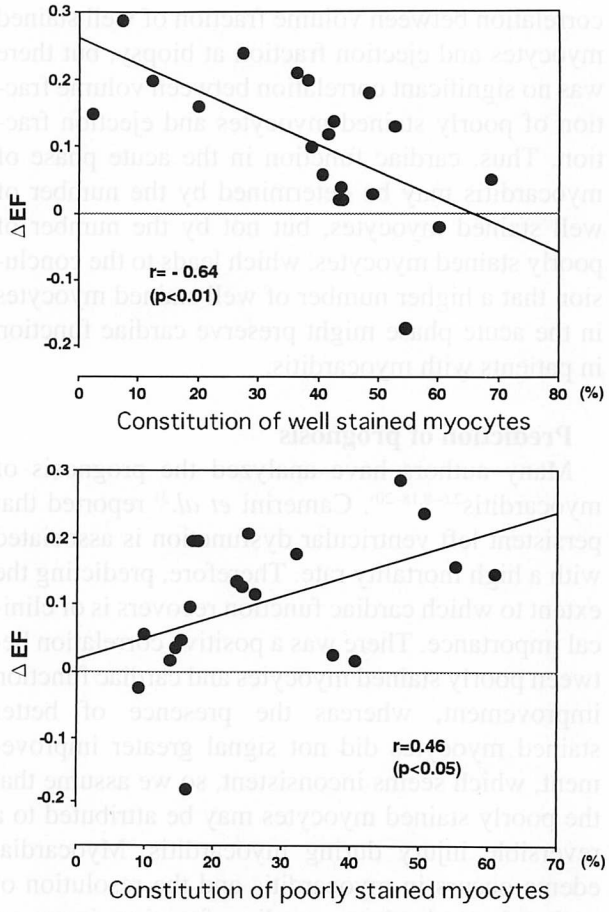


**Fig. 3** Correlation between the volume fraction of well stained myocytes (*upper*) and poorly stained myocytes (*lower*), and the EF improvement (ΔEF)

The volume fraction of well stained myocytes had a strong negative correlation ($r=-0.64$, $p<0.01$) with ΔEF. The volume fraction of poorly stained myocytes showed a close positive correlation ($r=0.46$, $p<0.05$) with ΔEF.

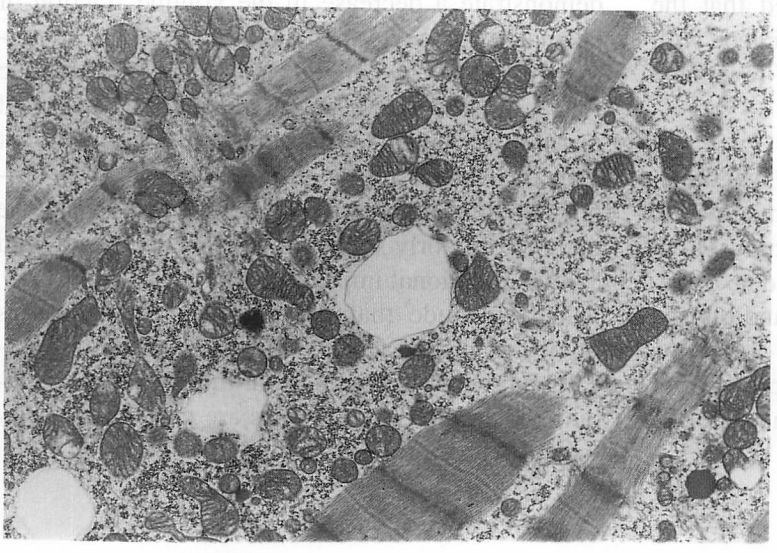

**Fig. 4** Electron micrograph showing swelling of myocytes with myofilament loss and swelling of mitochondria with focal loss of cristae only in the poorly stained myocytes (×9,000)

correlation between volume fraction of well stained myocytes and ejection fraction at biopsy, but there was no significant correlation between volume fraction of poorly stained myocytes and ejection fraction. Thus, cardiac function in the acute phase of myocarditis may be determined by the number of well stained myocytes, but not by the number of poorly stained myocytes, which leads to the conclusion that a higher number of well stained myocytes in the acute phase might preserve cardiac function in patients with myocarditis.

**Prediction of prognosis**

Many authors have analyzed the prognosis of myocarditis[2,6–8,18–20]. Camerini *et al.*[3] reported that persistent left ventricular dysfunction is associated with a high mortality rate. Therefore, predicting the extent to which cardiac function recovers is of clinical importance. There was a positive correlation between poorly stained myocytes and cardiac function improvement, whereas the presence of better stained myocytes did not signal greater improvement, which seems inconsistent, so we assume that the poorly stained myocytes may be attributed to a reversible injury during myocarditis. Myocardial edema occurs in myocarditis and the resolution of such edema leads to cardiac function improvement[21,22]. Therefore, we suspected that the presence of poorly stained myocytes may suggest a return to normal myocytes during the healing process of myocarditis, eventually resulting in a good prognosis. Although electron microscopic findings of the poorly stained myocytes in our study did not necessarily show hydropic degeneration[23], which is designated reversible injury, we speculated that the poorly stained myocytes were associated with hydropic degeneration in the process of reversible injury.

**Time course of myocarditis and cell fractions**

Endomyocardial biopsies were performed within 12 months of onset of symptoms in our study. However, analysis of each cell fraction showed no correlation between time of onset of illness and biopsy, nor between biopsy and maximal change of ejection fraction. It is therefore unlikely that the presence of poorly stained myocytes implies recent onset of myocarditis or reflects recovery time.

**Study limitations**

**Technical limitation of endomyocardial biopsy**

The biopsy samples were small (1 to 2 mm) and taken from one area of the heart (the left ventricular posterolateral free wall). We obtained three to four biopsy samples from several areas of the posterolateral wall to provide a representative sample of the histopathology, but nonetheless a very focal process could have been missed.

**Diagnostic problems**

Inconsistency between clinical and histological diagnosis of myocarditis has been studied[9]. At our institution, 20 of 25 (80%) patients with clinical myocarditis were also diagnosed with pathological myocarditis, and 20 of 37 (54%) patients with pathological myocarditis also had diagnoses of clinical myocarditis. Although there was significant compatibility ($p<0.05$) between the clinical and pathological diagnoses, there was also inconsistency in some patients. Therefore, we selected only those patients with both clinical and pathological diagnoses of myocarditis referred to as the Dallas Criteria[24].

**Sampling variability**

Schwarz *et al.* analyzed the sampling variability of endomyocardial biopsies in the point counting method[14]. They measured the volume fraction of histological findings in sections that were selected at random, but we measured all sections of one slice of each biopsy specimen to minimize sampling variability.

**Follow-up biopsy**

To prove our hypothesis, follow-up biopsy must demonstrate a decrease in the volume fraction of edematous myocytes and an increase in that of normal myocytes in patients with improved cardiac function. However, a second biopsy was performed in only two patients. Therefore, we did not attempt to analyze our results as a time sequential study. Biopsy findings in those two patients revealed that the number of poorly stained myocytes decreased with cardiac functional improvement.

We conclude that the presence of edematous myocytes in the acute phase of myocarditis indicates a favorable prognosis for improvement of cardiac function. Further studies are needed to clarify the mechanism for this finding.

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要　約

## 急性心筋炎の生検標本における染色性の濃淡は慢性期の心機能予測を反映する

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熊谷　和明　梅本　清嗣　山田　貴久　金銅　伸彦
南野　哲男　虎頭　簾　伯耆　徳武

今回我々は，心筋生検により得られた組織の標本から，慢性期の心機能を予測できるか否かを検討した．

臨床的に心筋炎と診断され，発症1年以内に左室心筋生検を施行し，組織学的にも心筋炎と診断された20例を対象とした．心機能は心エコー図により評価した．左室から採取しAzan-Mallory染色を施行した心筋生検標本を用い，染色の濃い心筋と淡い心筋の占める割合をそれぞれ定量的に算出した(ポイントカウント法)．

心筋生検より1年以内の駆出率の改善と染色の淡い心筋の占める割合との間には$r=0.46$，$p<0.05$の有意な正の相関を認めた．また，心筋生検時の駆出率と染色の濃い心筋の占める割合との間には$r=-0.64$，$p<0.01$の有意な負の相関を認めた．心筋炎において，心筋生検標本の染色度の定量的評価により，心機能の改善を予測できることが示唆された．

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