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Kaito, Hiroshi ; Nozu, Kandai ; Iijima, Kazumoto ; Nakanishi, Koichi ; Yoshiya, Kunihiro ; Kanda, Kyoko ; Rafal Przybysław Krol ; Yoshikawa, ...

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The Effect of Aldosterone Blockade in Patients with Alport Syndrome

Hiroshi Kaito, M.D.¹⁾, Kandai Nozu, M.D., Ph.D.¹⁾, Kazumoto Iijima, M.D., Ph.D.²⁾,
Koichi Nakanishi, M.D., Ph.D.³⁾, Kunihiro Yoshiya, M.D., Ph.D.⁴⁾, Kyoko Kanda, M.D.¹⁾,
Rafal Przybyslaw Krol, M.D.¹⁾, Norishige Yoshikawa, M.D., Ph.D.³⁾,
and Masafumi Matsuo, M.D., Ph.D.¹⁾

1) Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan

2) Department of Nephrology, National Center for Child Health and Development, Setagaya, Tokyo, Japan

3) Department of Pediatrics, Wakayama Medical University, Wakayama City, Wakayama, Japan

4) Department of Nephrology, Hara Urology Hospital, Kobe, Hyogo, Japan

Address for correspondence:

Kandai Nozu, M.D., Ph.D.,

Assistant Professor

Department of Pediatrics

Kobe University Graduate School of Medicine

Kusunoki-cho 7-5-1, Chuo-ku, Kobe, Hyogo, 6500017, Japan

Fax: +81-78-382-6099

Phone: +81-78-382-6090

E-mail: nozu@med.kobe-u.ac.jp

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Abstract

Recent studies indicate that adding the mineralocorticoid receptor antagonist spironolactone (SP) to angiotensin converting enzyme inhibitors (ACEI) or ACEI and angiotensin receptor blocker (ARB), which is known as a triple blockade, enhances the more beneficial effects on urinary protein excretion of patients with chronic kidney diseases. In this study, we explored the effects of SP on urinary protein excretion in patients with Alport syndrome featuring persistent proteinuria in spite of the long-term use of ACEI (lisinopril) or both ACEI and ARB (candesartan). **Five** patients with Alport syndrome were enrolled and SP treatment (25 mg/day) was started. At the start of SP administration, all patients showed good renal function and none of them suffered from hypertension. We decided to assess the effect of SP by determining the morning urinary protein/creatinine ratio (U-P/C) and estimated glomerular filtration rate (EGFR). After SP treatment was started, U-P/C was significantly reduced at 3, 6, 12 and 18 months, while EGFR did not change. The drop in systolic and diastolic blood pressure was statistically significant and serum potassium level was slightly elevated. None of the patients showed signs of severe hyperkalemia (> 5.0 mEq/L). These results suggest that aldosterone receptor blockade combined with ACEI and ARB therapy offers a valuable adjuvant treatment for the reduction of proteinuria in patients with Alport syndrome as in those with other chronic kidney diseases. SP can thus be expected to constitute a good renoprotective agent for Alport syndrome. These preliminary data indicate that large-scale trials of this therapy should be done.

Introduction

Alport syndrome, a progressive and hereditary form of glomerular nephritis, is a primary basement membrane disorder arising from mutations in genes encoding several members of the type IV collagen protein family [1]. It is a genetically heterogeneous disease with X-linked, autosomal recessive, and autosomal dominant variants. No specific curative therapy exists yet for this disorder, and most treatments are aimed at slowing the progression of the disease. Recently, using cyclosporine has been reported to show beneficial effects for human Alport patients and in animal models [2, 3, 4]. In addition, other studies have indicated that angiotensin converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) show antiproteinuric effects for patients with Alport syndrome and in animal models [5-10]. However, these agents are only temporally effective for reducing urinary protein excretion. Aldosterone is the principal physiological mineralocorticoid and acts to promote unidirectional sodium and water transport. Evidence has been recently increasing that aldosterone-induced vasculopathy may underlie progressive renal diseases [11, 12]. In fact, some studies show that adding spironolactone (SP), a

mineralocorticoid receptor antagonist, to ACEIs enhances the beneficial effects on urinary protein excretion in patients with chronic nephritis including diabetic nephropathy [13-16].

In this study, we explored the effect of SP on urinary protein excretion and the GFR in patients with Alport syndrome featuring persistent proteinuria despite the use of ACEI or a combination of ACEI and ARB.

Patients and methods

Patients

Table 1 summarizes the patients' characteristics at the start of SP therapy. **Five** patients with Alport syndrome were enrolled, three of whom had the X-linked inherited and two the autosomal dominant form. All patients satisfied the following modified Kashtan criteria [17]: (1) continuous microhematuria and proteinuria; (2) family history of one or several family members affected by well-documented Alport syndrome; (3) electron microscopic findings of renal biopsy showing pathognomonic lesions of the glomerular basement membrane (GBM), that is, thickening of the **GBM** accompanied by splitting and splintering of the lamina densa [1, 18-20]. Although Patient 5 was diagnosed as thin basement membrane disease, the electron microscopic GBM findings showed regional thickening and splintering abnormalities and both the patient and her father had persistent proteinuria. We therefore assumed that she was **Alport syndrome in the early stage**. When the SP treatment started, the patients were between 11 and 19 years of age (median 18.0 years) and all had persistent proteinuria, with a morning urinary protein/creatinine ratio (U-P/C) of more than 0.2 **g/g** (median 0.61 **g/g**) in spite of treatment with ACEI (lisinopril) or ACEI and ARB (candesartan) combined for more than 19 months. Four patients were treated with both ACEI and ARB and the other with ACEI only because of acute renal failure 2 months after the start of ARB treatment, when the serum creatinine level had increased from 0.96 to 4.24 mg/dl. All patients were normotensive and estimated glomerular filtration rates (EGFRs) were above 90ml/min. One patient was treated with pravastatin for hyperlipidemia, and another patient with allopurinol for hyperuricemia. Final average doses of lisinopril and candesartan were 0.20 ± 0.05 mg/kg/day (11.0 ± 2.45 mg/day) and 0.11 ± 0.03 mg/kg/day (5.5 ± 0.96 mg/day), respectively.

Methods

The patients visited the outpatient clinic in Kobe University Hospital at least once every 3 months. At each visit, blood pressure was measured with an automatic sphygmomanometer and morning urine was examined. We decided to assess the effectiveness of SP in terms of U-P/C and EGFR [21-25]. Blood samples were taken for serum creatinine, albumin and potassium measurements with standard laboratory

techniques. **During treatment, no patients were instructed on special dietary therapy such as potassium restriction.** EGFR was calculated with Schwartz's formula: $EGFR = k \times \text{height (cm)} / \text{serum creatinine (mg/dl)}$ (k : **0.55 for boys of 2-12 years and also girls of 2-21 years, and 0.70 for males of 13-21 years years**) [24, 25]

Study Design

Patients with proven Alport syndrome and with persistent proteinuria in spite of ACEI or combined ACEI and ARB treatment were invited to participate in this clinical study after information had been given about the natural history of the disease, and the possibility of reducing urinary protein excretion as well as slowing down disease progression with SP treatment. Parents of the patients gave their informed consent. The dosage of SP was fixed at 25mg a day, which was based on the previous studies [14, 15]. U-P/C just before the start of SP administration was compared with U-P/C at 3, 6, 12, and 18 months after the start of the treatment and baseline EGFR was compared with that at 18 months. Blood pressure and serum potassium levels before the treatment were compared with those **three** months after the start of treatment.

Statistical analysis

Data are expressed as mean \pm SE. Statview®-J5.0 software was used for statistical calculations. Changes in parameters before and after treatment were compared by using two-group paired t tests, and P values of $< .05$ were considered significant.

Results

Proteinuria

Fig. 1 shows the time course changes in U-P/C. Before the start of SP administration, U-P/C was 0.61 ± 0.10 g/g and showed no significant reduction, that is, the maximal antiproteinuric effects obtained with ACEI or both ACEI and ARB were no longer observed. After the start of the treatment with SP, U-P/C was significantly reduced at every time point ($P < .05$) and gradually dropped to a minimum (0.13 ± 0.06 g/g) at 18 months.

Estimated GFR

EGFR remained virtually unchanged throughout SP therapy (before SP treatment: 129.2 ± 21.4 ml/min; 18 months later: **110.1 ± 30.1 ml/min; $P=0.115$**) (Fig. 2).

Blood Pressure

Before SP was initiated, the systolic and diastolic blood pressure values were 110.8 ± 5.0 mmHg and 58.2 ± 2.7 mmHg. Three months after SP administration, they had dropped to 91.2 ± 6.6 mmHg and 51.0 ± 3.0 mmHg, respectively (Table 2). In spite of this significant reduction ($P < .05$), none of the patients

complained of dizziness or orthostatic hypotension.

Serum potassium

Although serum potassium level 3 months after SP treatment was significantly higher than that before the start of treatment ($P < .05$), none of the patients developed serious hyperkalemia (> 5.0 mEq/L) as result of the SP therapy (Table 2).

Adverse Events

There were no adverse events, including liver dysfunction, allergic manifestations, hyponatremia, arrhythmia, muscle weakness, or gynecomastia. No patients dropped out of this study.

Discussion

In this study, we demonstrated that treating Alport patients with SP in addition to ACEI or ACEI and ARB is helpful for reducing urinary protein excretion.

Alport syndrome is bound to progress to end-stage renal disease and there is no treatment **except** dialysis and transplantation [1, 17, 18, 19]. Callis *et al.* reported that cyclosporine may halt progression in human Alport syndrome with severe proteinuria [3, 4], and this was followed by a demonstration that cyclosporine had beneficial effects in terms of improving renal function and delaying the onset of renal failure in canine X-linked Alport syndrome [2]. This study concluded that cyclosporine may be able to alter glomerular hemodynamics, resulting in a delayed deterioration of glomerular basement membrane. On the other hand, another study found that cyclosporine therapy for Alport syndrome not only suppressed urinary protein excretion but **accelerated** renal fibrosis and resulted in reduced GFR [26]. It is thus very difficult to determine how long cyclosporine therapy should be continued and how many doses of cyclosporine are appropriate for Alport syndrome. Grodecki *et al.* used ACEI to treat Samoyed dogs with X-linked hereditary nephritis and found the treatment beneficial with respect to renal function, renal structure, and survival [5]. The affected treated dogs showed a significant transient reduction in glomerular basement membrane splitting. As for the effectiveness of ARB for Alport syndrome, Inoue *et al.* demonstrated in a mouse model that ARB contains more renoprotective **properties** than calcium antagonists, thus inhibiting the progression of glomerulopathy and interstitial fibrosis [10]. Some studies have described the effect of ACEI on patients with Alport syndrome [8, 9] and now it is believed that it is more effective to treat them with a combination of ACEI and ARB than with either agent alone. We also used treatment with ACEI and ARB, but found it to be temporarily effective and not effective enough to keep urinary protein excretion levels reduced in the long term.

Chrysostomou *et al.* reported that SP was effective in long-term ACEI therapy for chronic kidney disease with persistent proteinuria [13]. This was followed by some reports which confirmed the

effectiveness of aldosterone receptor blockade for reducing urinary protein excretion [14-16]. A recent, double-blind, placebo-controlled study indicated that SP combined with ACEI had an antiproteinuric effect on chronic kidney diseases including diabetes nephropathy and IgA nephropathy [27].

Aldosterone is a major regulator of extracellular fluid volume and the major determinant of potassium metabolism. In recent years, evidence has been accumulating that aldosterone can affect vascular remodeling and collagen formation, and exerts a non-genomic action to modify endothelial function. It can also produce deleterious effects in both the cardiovascular system and the kidneys [11, 12]. Indeed, there has been considerable experimental and clinical evidence that aldosterone can contribute to the development of nephrosclerosis and renal fibrosis in models of diabetes and hypertension [28, 29]. In addition, a number of studies using human and animal models have indicated that aldosterone blockade protects against progression of end-organ damage through both hemodynamic and direct cellular actions. Nishiyama *et al.* demonstrated in rats that aldosterone caused mesangial cell proliferation and urine protein excretion while activating the mitogen-activated protein kinase (MAPK) pathway, and this deterioration improved with the administration of eplerenone, a selective aldosterone receptor antagonist [30]. Aldosterone has been shown to stimulate type IV collagen production, the principal component of glomerular basement membrane and mesangial matrix in cultured rat mesangial cells, and treatment with a combination of ACEI and SP to reduce urinary type IV collagen excretion in patients with diabetic nephropathy [31]. SP may thus be able to attenuate inflammatory changes in renal parenchyma of Alport patients with continuous urine protein excretion.

Hyperkalemia is potentially one of the most serious complications resulting from combining SP with ACEI and ARB. Since the randomized aldosterone evaluation study (RALES) was published, hyperkalemia-associated morbidity and mortality as well as the rate of prescriptions for SP have increased [32]. The patients in our study were carefully screened for risk of developing hyperkalemia as a result of the combined treatment. While the serum potassium level after treatment with SP was significantly higher than before, it was in no way a serious clinical problem. **Normal estimated GFRs of all patients may prevent serum potassium levels from being much more higher, and this** means that SP treatment in our study could exert a renoprotective effect without severe adverse events. Moreover, the suppression of aldosterone activity with ACEI, ARB and SP was quite successful, and resulted in only mild hyperkalemia.

We have demonstrated that the addition of SP to ACEI only or to ACEI and ARB resulted in a reduction of U-P/C after three months in patients with Alport syndrome, and that this reduction was maintained for as long as 18 months. The systolic and diastolic blood pressure dropped significantly after treatment with SP. Although maintenance of adequate blood pressure will often cause a reduction in

urinary protein excretion, all the patients in our study maintained normotension and good renal function even before SP treatment, so it can be concluded that a reduction in the blood pressure is not a direct factor in the reduction of urinary protein excretion.

Our study has certain limitations. The first is that this is an uncontrolled and small-scale study with only five patients enrolled. Moreover, it is quite unusual two out of five patients to be genetically autosomal dominant variants, for most forms of the disease are generally of the X-linked variety and the autosomal dominant form accounts for only a small percentage [1, 18]. In addition, electron microscopic examination resulted in one of the patients being diagnosed as thin basement membrane disease with partial abnormalities of the glomerular basement membrane. However, it is very difficult to discriminate between the initial stage of Alport syndrome and thin basement membrane disease, and since both the patient and her father have continuous proteinuria as well as microhematuria, we have now evaluate her renal biopsy findings as the initial stage of Alport syndrome. This indicates that the Alport-related gene should be analyzed in these patients [1, 19]. The second shortcoming is that this is a rather short-term study. Although it is generally accepted that lowering protein excretion is a desirable treatment goal for chronic proteinuric kidney disease, it could not be determined in this study whether treatment with SP can suppress urinary protein excretion for a long time. In this connection, we previously found that urinary protein excretion increased again during long-term ACEI therapy.

In summary, we were able to demonstrate that adding SP to ACEI and ARB may be effective for lowering proteinuria in Alport syndrome. However, a larger-scale study with longer follow-up is required to demonstrate whether the results shown in our study translate to improved renal survival for patients with Alport syndrome.

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Figure Legends

Fig. 1 Proteinuria in patients treated with SP for 18 months

Proteinuria expressed as morning urinary protein/creatinine ratio (U-P/C). Before the start of spironolactone (SP) therapy, U-P/C showed no significant reduction, but was significantly reduced 3, 6, 12 and 18 months after the start of treatment with SP. * $P < .05$

Fig. 2 Changes over three months in estimated glomerular filtration rates (EGFRs) of individual patients pre- and post-treatment with spironolactone

EGFR remained unchanged throughout SP therapy.

Table 1 Characteristics of five patients at the start of SP treatment

Patient	1	2	3	4	5
Sex (M/F)	M	M	M	M	F
Age (years)	11	18	19	13	17
Mode of inheritance	XL	XL	XL	AD	AD
Period of medication (ACEI and ARB; months)	84	54*	19	80	32
Drugs used concomitantly	No	allopurinol	No	pravastatin	No
Blood pressure (mmHg)	118/50	116/58	114/66	115/61	91/56
EGFR (ml/min)	106.8	94.3	121.8	213.2	110.1
U-P/C (g/g)	0.48	0.68	0.75	0.85	0.28
Serum creatinine (mg/dl)	0.49	1.06	0.76	0.35	0.57

*Only ACEI was prescribed because of the acute renal failure with ARB.

XL: X-linked, AD: autosomal dominant, U-P/C: morning urinary protein/creatinine ratio, EGFR: estimated glomerular filtration rate

Table 2. Changes in blood pressure and serum potassium as a group in pre and post-treatment with SP for 3 months. Data represent mean \pm SE.

	Before SP treatment value	Three months after SP treatment	P
Blood pressure (mmHg)			
Systolic	110.8 \pm 4.99	91.2 \pm 6.55	0.016
Diastolic	58.2 \pm 2.65	51.0 \pm 2.97	0.009
Serum potassium (mEq/L)	4.10 \pm 0.12	4.42 \pm 0.10	0.045

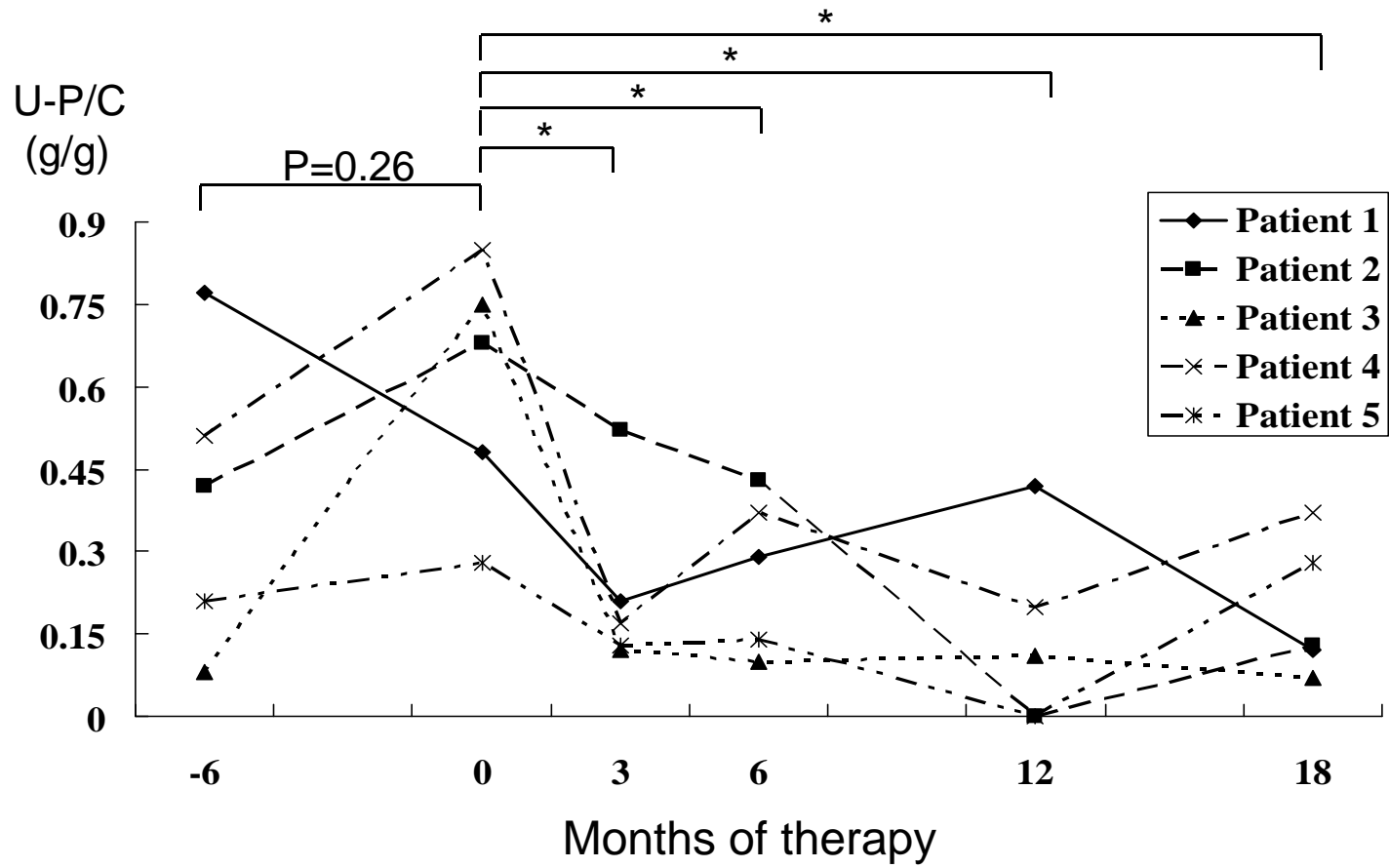


Fig.1

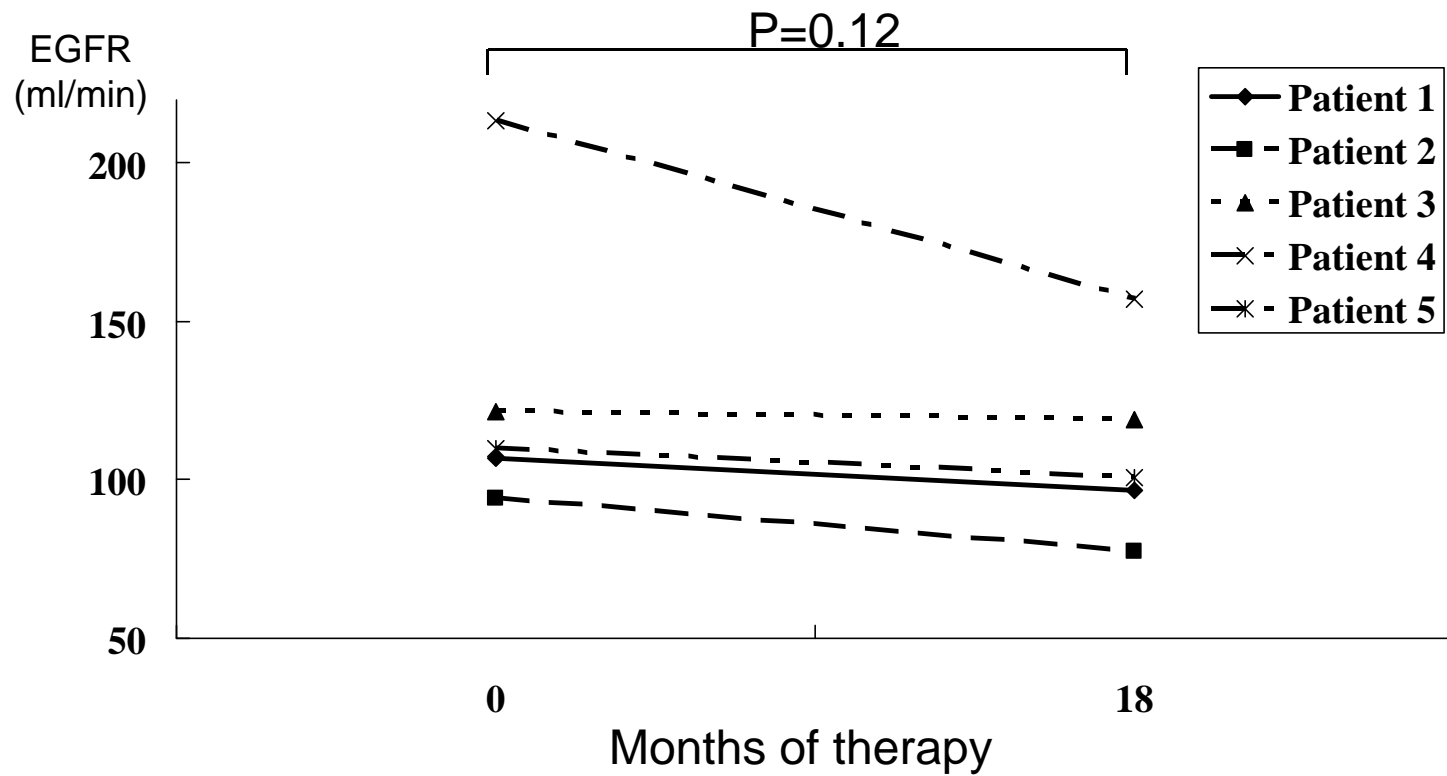


Fig. 2