

Routine blood monitoring in acne vulgaris patients on isotretinoin: Is reassessment warranted?

A retrospective cohort study from Tygerberg Hospital

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Background. Oral isotretinoin is an indispensable treatment for patients with resistant and severe acne vulgaris, both in South Africa (SA) and globally. While routine laboratory monitoring is often deemed unnecessary for young, healthy patients, it remains unclear whether this applies in the SA context.

Objectives. To assess the need for routine blood monitoring for acne vulgaris patients on isotretinoin treatment at Tygerberg Hospital, SA. Specifically, we wanted primarily to determine the prevalence of patients with acne vulgaris whose oral isotretinoin treatment was altered owing to blood result abnormalities, and secondarily, to determine the prevalence of adverse events and liver function and lipid profile abnormalities associated with isotretinoin.

Methods. This was a retrospective cohort study of hospital records from patients with acne vulgaris treated with oral isotretinoin at the dermatology clinic at Tygerberg Hospital between 1 January 2020 and 31 December 2023. There were 89 eligible records extracted from the Tygerberg Hospital Enterprise Content Management system. Baseline and 6-week follow-up laboratory data, including liver function tests and lipid profile, were extracted retrospectively from the National Health Laboratory Service.

Results. The sample comprised 89 patient hospital records, 62% male and 38% female. Of the total, 53% were aged between 12 and 20 years. Blood result abnormalities led to alterations in treatment regimens for 2/89 (2.2%) patients: one patient required treatment termination, while another continued on low-dose isotretinoin instead of the planned dose increase. Two patients (2.2%) developed increased aspartate aminotransferase levels and five (5.6%) developed new alanine aminotransferase increases above the upper limit of the normal range. Elevated triglyceride levels occurred in two patients (2.2%), and elevated cholesterol levels in nine (10.1%). Adverse events resulting in the premature termination of treatment with isotretinoin included one case of severe chest pain, one patient with pseudotumour cerebri and one patient who developed exuberant granulation tissue.

Conclusion. This single-centre retrospective review highlights the scarcity of blood result abnormalities due to isotretinoin in patients with acne vulgaris. There were only two patients (2.2%) who had their management altered owing to blood result abnormalities, and both had chronic conditions. This may suggest that routine monitoring is indicated in patients with comorbidities, but not in otherwise healthy young patients with acne. However, larger prospective studies are needed in SA before such conclusions can be drawn.

Keywords: acne vulgaris, isotretinoin, blood monitoring

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Oral isotretinoin is an essential and indispensable treatment for patients with resistant and severe acne vulgaris, both in South Africa (SA) and globally.^[1,2] It has been suggested that routine laboratory monitoring may not be warranted for young, healthy patients.^[3,4] While this would reduce the burden on patients and would be a welcome cost-saving measure, especially in resource-limited areas, saving approximately ZAR388/USD21.70 per patient, it should only be considered if patient safety remains uncompromised.^[5,6]

Acne vulgaris is defined as a chronic inflammatory dermatosis that is notable for open and/or closed comedones (blackheads and whiteheads) and inflammatory lesions including papules, pustules and nodules.^[7] The four major pathogenic factors associated with acne vulgaris are: (i) follicular hyperkeratinisation; (ii) androgens causing excess sebum production; (iii) proliferation of the organism *Cutibacterium acnes*; and (iv) immunological and inflammatory responses.^[2,8,9] Acne

vulgaris usually presents during puberty, and disappears in a patient's early twenties.^[10]

Clinical guidelines for the management of acne vulgaris, published in the *South African Medical Journal* in 2005,^[2] are still widely followed in SA. These guidelines grade acne based on the predominant lesion present on the skin, with treatment tailored accordingly. Grade 3 - 4 acne vulgaris is considered severe, and is characterised by pustules, nodules and cysts.^[2] The Tygerberg Hospital Dermatology Department (Cape Town, SA) uses an internal guideline based on the acne guideline update in the *SAMJ*, added as [Appendix 1](#).^[2]

Initial treatments often include benzoyl peroxide, topical retinoids, topical antibiotics and azelaic acid, with oral contraceptives being an option for women.^[2] If these do not yield improvement, tetracycline-based antibiotics are typically prescribed for 3 months. For cases where there is no response or the acne is severe, oral isotretinoin is recommended.^[2] Despite its effectiveness, isotretinoin is associated with multiple

potential side-effects and complications, with teratogenicity being among the most significant concerns.^[11] Other potential side-effects include xeroderma, as well as possible psychiatric, gastrointestinal and neurological symptoms.^[12]

Currently, patients at Tygerberg Hospital who require isotretinoin initiation have routine blood tests consisting of a full blood count, lipid profile and transaminases to monitor for any potential side-effects to isotretinoin and to guide isotretinoin dosing. Additionally, all women of child-bearing age undergo formal or rapid pregnancy tests, which remain an imperative part of the work-up before isotretinoin initiation. There have been numerous studies over the past two decades investigating whether monthly blood monitoring when using isotretinoin is necessary.^[3,4,11-16] Severe adverse events reported with isotretinoin are rare (<1 in 10 000), and might not be prevented by routine laboratory monitoring. As such, routine laboratory monitoring may be an unnecessary inconvenience for patients and a waste of healthcare expenditure.^[3] Implementing cost-saving measures without compromising patient safety would be beneficial, particularly in a lower middle-income country such as SA.^[5,6]

Objectives

The aim of the study was to assess the need for routine blood monitoring for acne vulgaris patients on isotretinoin treatment at Tygerberg Hospital. We wanted primarily to determine the prevalence of patients with acne vulgaris whose oral isotretinoin treatment was altered owing to blood result abnormalities, and secondarily, to determine the prevalence of adverse events and liver function and lipid profile abnormalities associated with isotretinoin.

Methods

This was a retrospective cohort study of hospital records from patients with acne vulgaris treated with oral isotretinoin at the dermatology clinic at Tygerberg Hospital. Records that were eligible for the study were those from patients who attended the clinic for the first time between 1 January 2020 and 31 December 2023. All records from new patients aged ≥ 12 years who received oral isotretinoin for the treatment of acne vulgaris were included in the study. Records of patients who were given isotretinoin for a diagnosis other than acne vulgaris, patients aged <12 years and those without any blood tests on the National Health Laboratory Service (NHLS) system were excluded.

The starting dose of isotretinoin was individualised, taking into account that male sex, younger age and greater severity of acne increase the risk of an acne flare when initiating treatment, which may be mitigated by a lower starting dose.^[18] A disproportionate number of severe acne cases is seen at Tygerberg Hospital given that it is a tertiary referral facility. For this reason, isotretinoin is often started at a lower dose, and prednisone is often added to mitigate the initial isotretinoin-induced flare.

Clinical records from the Tygerberg Hospital Enterprise Content Management system and blood results from the NHLS were reviewed. The data were extracted to a REDCap (Research Electronic Data Capture) database. The collected information included basic demographic data, previous medical history, previous acne vulgaris treatment, initiation dose and then adjusted dose of isotretinoin, as well as the baseline and follow-up blood test results. The data had no patient identifiers, were anonymised prior to analysis and were password protected. As per the Tygerberg Hospital Department of Dermatology protocol, the routine blood tests include baseline full blood count, baseline and follow-up total cholesterol, triglycerides, aspartate transferase (AST) and alanine transaminase (ALT) levels. All side-effects and reasons for dose adjustment or premature termination of treatment, if applicable, were recorded.

Ethical clearance was obtained from Tygerberg Hospital as well as the Health Research Ethics Committee of Stellenbosch University (ref. no. S23/11/309).

Stata version 18 (StataCorp, USA) was used for descriptive data analysis. Any patient who had one of the four blood result parameters above the upper limit of normal, as defined by the NHLS, at follow-up was considered to have an abnormal result. Patients were categorised according to whether their isotretinoin dose was subsequently maintained, increased or decreased, or treatment prematurely terminated. No inferential statistics were applied.

Results

The study retrospectively reviewed the medical records of 196 patients diagnosed with moderate-to-severe or severe acne vulgaris at Tygerberg Hospital, Cape Town, SA, between 1 January 2020 and 31 December 2023. Of the 196 records, 90 patients were started on isotretinoin. One patient did not have any blood tests, resulting in a final sample size of 89. The number of new patients per year who started isotretinoin ranged from 22 to 27 between 2021 and 2023. In 2020, only 16 new patients were seen, likely owing to the COVID-19 pandemic.

The eligible population comprised 55 males (61.8%) and 34 females (38.2%). Most patients (52.8%, $n=47$) were aged between 12 and 20 years, followed by 33.7% ($n=30$) aged 21 - 30 years, and 13.4% ($n=12$) aged 31 - 40 years. The mean (standard deviation) age was 21.8 (6.95) years. The age and sex distribution by year are summarised in Table 1.

Acne vulgaris treatments received prior to starting oral isotretinoin are shown in Fig. 1. Regarding previous topical treatment, 15 patients had been on a topical retinoid, 10 had been on benzoyl peroxide and 15 had been on a fixed combination of topical adapalene 0.1% and benzoyl peroxide 2.5% (Epiduo). Regarding previous oral treatments, 17 had been on an oral contraceptive, and 67 had been on doxycycline, of whom 54% were treated for between 2 and 4 months, 4% for <2 months, 22% for >4 months and 20% for an unknown duration. Thirty-eight percent of the patients who were started on isotretinoin were also initially

Table 1. Demographics of study participants (N=89)

Age, years	2020		2021		2022		2023		M, total	F, total	Total
	M	F	M	F	M	F	M	F			
12 - 20	6	3	8	4	12	4	8	2	34	13	47
21 - 30	5	2	5	3	2	2	5	6	17	13	30
31 - 40	0	0	3	4	0	2	1	2	4	8	12
Total	11	5	16	11	14	8	14	10	55	34	89

M = male; F = female.

treated with prednisone (Fig. 2). Doses of prednisone ranged from 10 mg to 40 mg daily, with duration ranging from 5 days to 1 month. The most frequent dosing regimen (mode) was 20 mg daily for 2 weeks (47.1% of patients receiving prednisone). Almost half (45%) of patients were started on an isotretinoin dose of 20 mg. The next most common dose was 40 mg (24%), then 30 mg (18%), and lastly 12% were started on 10 mg.

Blood results are shown in Table 2. In summary, a total of 16 baseline and 19 follow-up blood test results were above the average reference range. Missing data accounted for 53/356 (14.9%) of the baseline and 111/356 (31.2%) of follow-up values. At baseline, elevated results were

observed in 6% for AST (median 53.5 U/L), 6.1% for ALT (median 55.0 U/L), 6.8% for triglycerides (median 1.92 mmol/L) and 2.5% for total cholesterol (median 6.33 mmol/L). At follow-up, elevated median (interquartile range (IQR)) results were noted in 4.3% for AST (42 (44) U/L); 7.9% for ALT (35 (6) U/L); 6.9% for triglycerides (2.45 (0.55) mmol/L); and 23.9% for total cholesterol (5.39 (0.98) mmol/L). Fig. 3 further further illustrates the number of patients with elevated follow-up blood results, showing patients treated with prednisone and those who did not receive prednisone.

Fig. 4 shows the treatment results of the 89 eligible patients in terms of their dosing schedule and number of side-effects, and

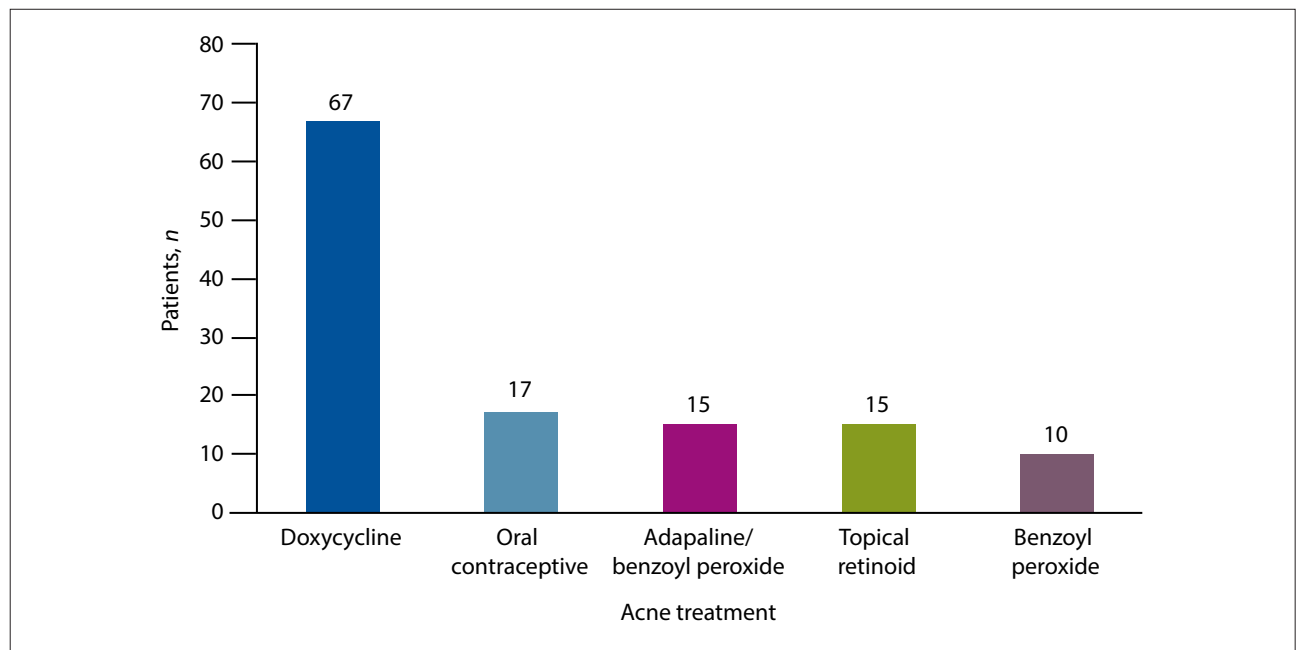


Fig. 1. Patients with acne vulgaris and previous treatments (N=124).

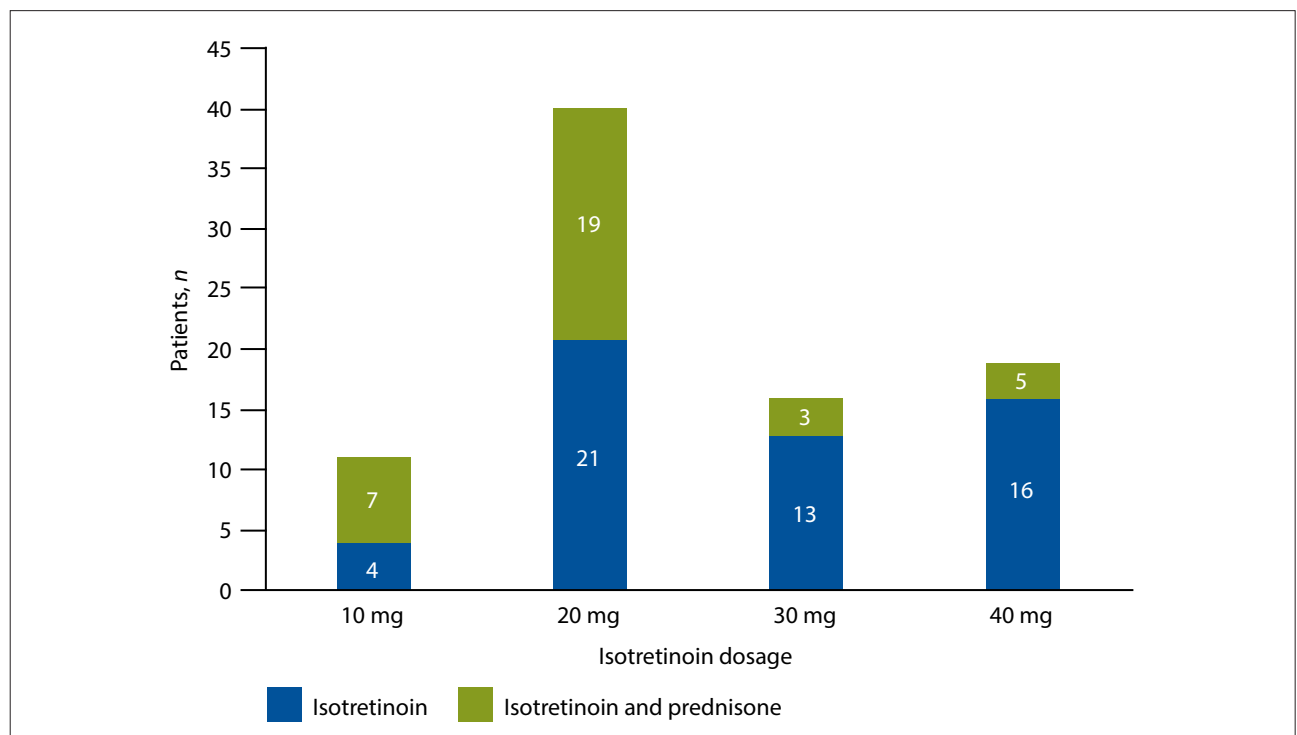


Fig. 2. Cumulative bar graph showing the number of patients using prednisone embedded in the number of patients per isotretinoin dose (N=88).

highlights which of these had management changes owing to blood results.

Thirty-six percent (n=32) of the sample reported experiencing side-effects. The most noted side-effects were xerosis (21%), mood-related symptoms (3%) and arthralgia (3%). Adverse events resulting in the premature termination of treatment with isotretinoin included one case of severe chest pain, one patient with pseudotumour cerebri (PTC) and one patient who developed exuberant granulation tissue. None of these patients had blood result derangements, except for the patient with PTC. This patient was noted to have an >300% increase in their ALT levels on follow-up; however, this was only 1.05 times the upper limit of the normal reference range. This patient also had a 27.7% increase in cholesterol level, which was only 1.2 times the upper limit of the normal reference range. Of the remaining 29 patients who experienced side-effects, 23 (79.3%) completed treatment, 4 (13.7%) were lost to follow-up, 1 (3.4%) ran out of medication and 1 (3.4%) had a drug-induced liver injury.

Only one (1.1%) patient had their treatment terminated owing to abnormal blood results. This patient was initially treated with doxycycline, then started on isotretinoin owing to a poor response. The patient was started on 20 mg isotretinoin daily, with the addition

of 20 mg of prednisone for the first week. Ultimately, treatment was terminated owing to a transaminitis at baseline, and therefore not as a result of a derangement caused by the oral isotretinoin. This patient had a significant comorbidity (HIV infection), and 1 month after isotretinoin their ALT level stayed the same and their AST level had decreased by 55%. However, treatment was stopped pending further investigation of liver dysfunction. There was one other patient where the blood results influenced the treatment decision on dose adjustment. This patient was started on 30 mg of isotretinoin and was not given prednisone. This patient also had a chronic condition (dyslipidaemia) and had recently started simvastatin. The patient was noted to have elevated triglycerides of 2.75 mmol/L and cholesterol of 7.0 mmol/L at baseline, but was then maintained on their initiation dose because of an elevated AST level of 48 U/L, 1.37 times the upper limit of normal. The AST had normalised on repeat blood tests done 6 months later, but the cholesterol level had significantly increased to 9.27 mmol/L, almost twice the upper limit of normal. Three (3.3%) additional patients were noted to have blood result abnormalities, but these results did not influence the treatment decision regarding dose adjustment. One of these patients was a 14-year-old who did not have their dose increased from the initiation dose of 0.5 mg/kg,

Table 2. Summary of baseline and follow-up blood tests (16 elevated baseline and 19 elevated follow-up results)

Test	Time point	Records with results, n	Elevated result, n (%)	Elevated result, median (IQR)	Normal result, n (%)	Missing result, n	Comment
AST (U/L)	Baseline	72	4 (6.0)	53.5	68 (94.0)	17	-
	Follow-up	70	3 (4.3)	42 (44)	67 (95.7)	19	-
ALT (U/L)	Baseline	81	5 (6.1)	55.0	76 (93.1)	8	-
	Follow-up	89	7 (7.9)	35 (6)	64 (92.1)	18	-
Triglycerides (mmol/L)	Baseline	73	5 (6.8)	1.92	68 (93.2)	16	-
	Follow-up	60 (58 valid)*	4 (6.9)	2.45 (0.55)	54 (93.1)	31	2 excluded (no baseline)
Total cholesterol (mmol/L)	Baseline	81 (77 valid)†	2 (2.5)	6.33	75 (97.5)	8	4 excluded (no follow-up)
	Follow-up	46	11 (23.9)	5.39 (0.98)	35 (76.1)	43	-

AST = aspartate transferase; ALT = alanine transaminase.
 Missing data: 53/356 baseline (14.9%) and 111/356 (31.2%) follow-up.
 *Excludes four that did not have a follow-up value.
 †Excludes two high measurements that did not have an initiation value.

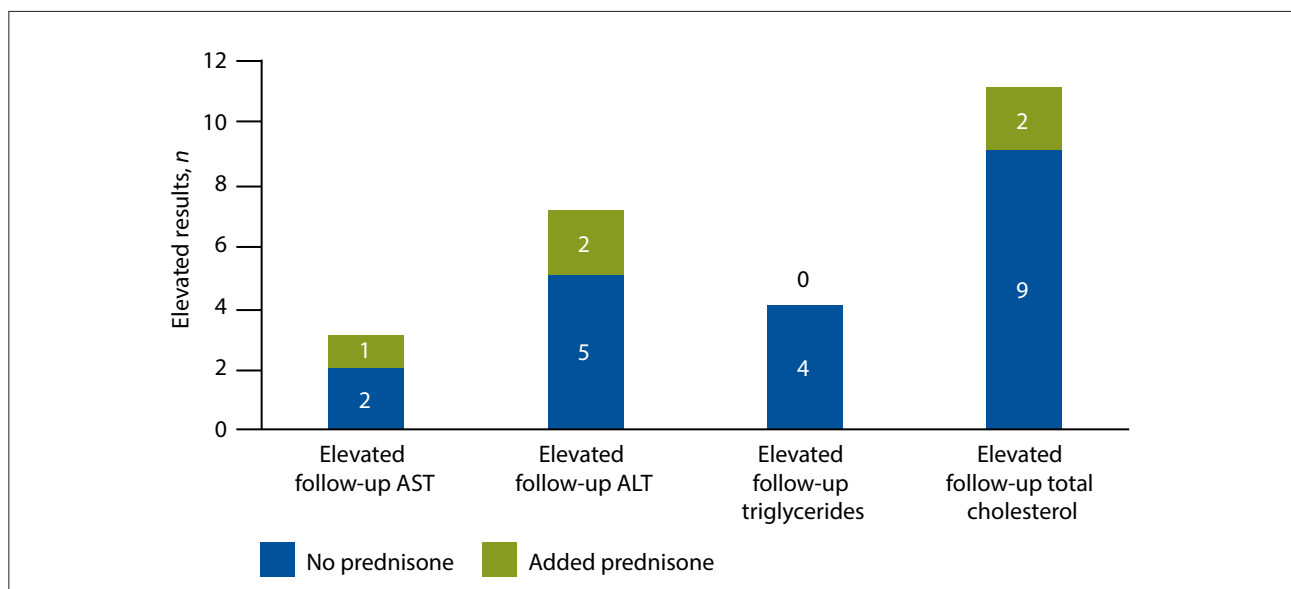


Fig. 3. Stacked bar graph showing raised blood levels by prednisone use (N=25). (AST = aspartate transferase; ALT = alanine transaminase.)

but had a 48% increase in AST with a new level of 37 U/L (1.16 times the upper limit of normal), a 138% increase in ALT with a new level of 31 U/L (1.55 times the upper limit of normal) and a 25% increase in total cholesterol with a new level of 6.16 mmol/L (1.23 times the upper limit of normal). The second patient experienced a 19% increase in total cholesterol without experiencing side-effects, and their initiation dose of just <0.5 mg/kg was maintained. The new level was 5.39 mmol/L, only 1.08 times the upper limit of normal. A third patient had a transiently elevated AST and ALT on initiation, which then normalised after a month on isotretinoin. The baseline levels were 49 U/L and 55 U/L, 1.23 times and 1.38 times greater than the upper limit of normal, respectively.

Discussion

The main finding of this study is that, over a 4-year period, one patient (1.1%) with a significant comorbidity (HIV infection) had baseline laboratory abnormalities resulting in withdrawal of intended isotretinoin treatment. No patients had follow-up laboratory abnormalities necessitating termination of treatment. Discontinuation of treatment is typically advised for severe elevations in transaminases, considered >3 times the upper limit of normal.^[17] In this case, the patient's ALT level was twice the upper limit of normal, and the AST was >4 times the upper limit of normal. Therefore, the treatment was appropriately terminated. This patient was asymptomatic at the time of treatment initiation and follow-up, so without these blood tests, the medication would have been continued. However, the follow-up blood results after 1 month of isotretinoin were mostly on a downward trend, as the AST more than halved, and the ALT

remained the same. This finding highlights that blood monitoring may be important in patients who have comorbidities that require drugs with hepatic metabolism, for example, HIV-positive patients on antiretrovirals. Furthermore, all patients should be informed of potential side-effects of isotretinoin with specific reference to hepatic dysfunction and pancreatitis, so that occurrence of relevant symptoms can prompt appropriate testing.

In one patient with dyslipidaemia on simvastatin, isotretinoin dosing was kept low (at ~0.5 mg/kg/day), rather than increased at the first follow-up visit as had initially been planned, owing to a minor elevation in transaminases. The patient had an AST of 1.37 times the upper limit of normal. Transaminase elevations due to isotretinoin are usually mild, and typically return to normal despite continuous therapy.^[19] With this in mind, these slight transaminase elevations may have returned to normal even if the dose was increased as planned. The three other patients who were noted to have incidental blood result abnormalities that did not affect their management had ALT values that were at most 1.6 times the upper limit of normal, and AST levels that were at most 1.2 times the upper limit of normal. The cholesterol elevations were at most 1.2 times more than baseline, and there were no triglyceride abnormalities.

The benefits of corticosteroid use in autoimmune hepatitis are well described. There is, however, a lack of evidence to support its use for drug-induced liver injury. This suggests that prednisone used in conjunction with the isotretinoin is unlikely to have masked any further transaminase elevations in patients, particularly given the short durations and low doses used.^[20]

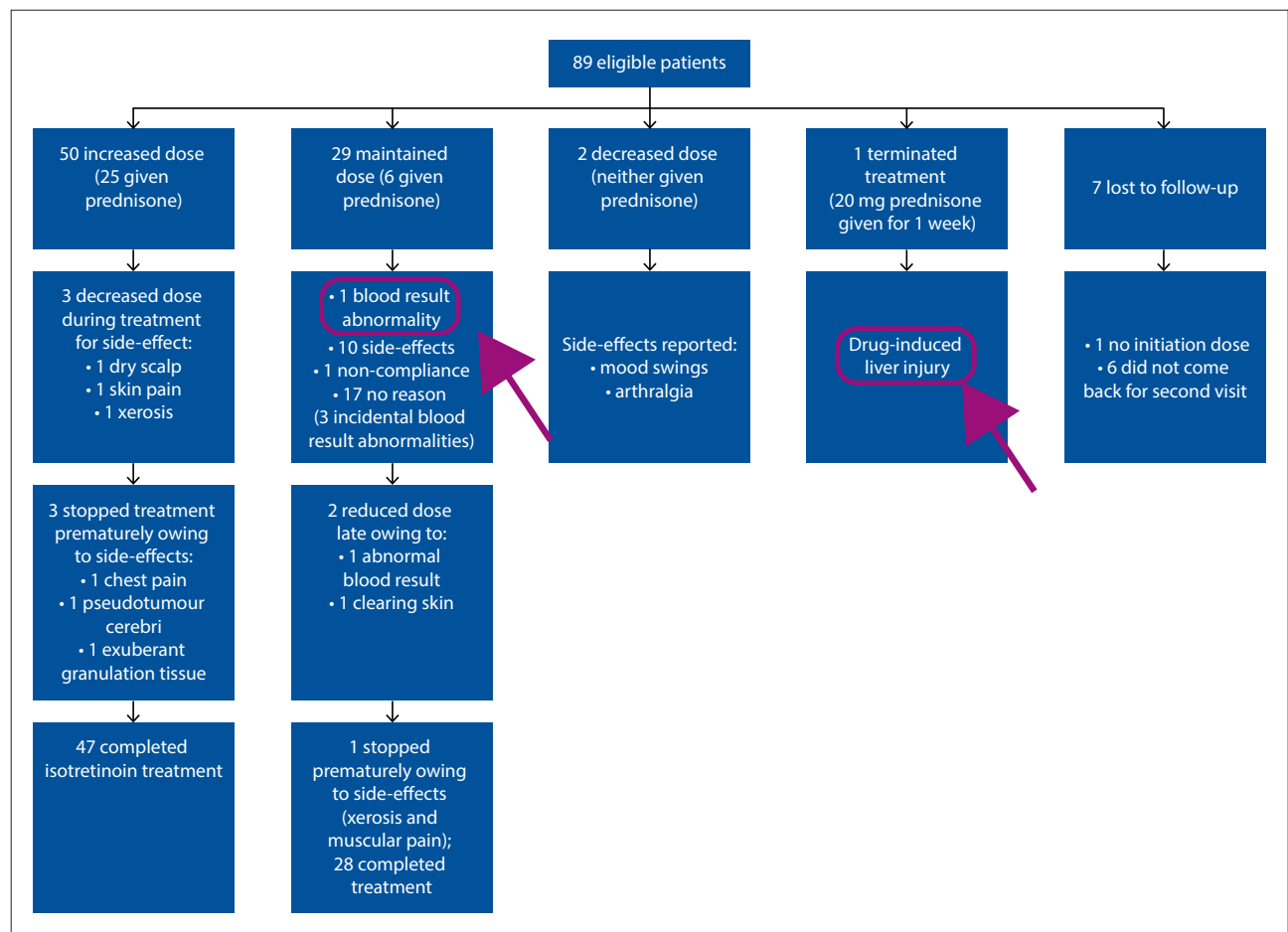


Fig. 4. Flow diagram showing different treatment strategies and outcomes of study participants.

The risk of pancreatitis underscores the need for careful management of elevated lipid levels.^[17] Over our 4-year study period, there were no cases of pancreatitis. It is suggested that triglyceride elevation during a short course of isotretinoin is a marker of atherosclerosis risk rather than a cause of atherosclerosis, but prolonged isotretinoin use in patients with underlying dyslipidaemia is not recommended.^[21] Further research could be done into whether having family members with dyslipidaemia puts one at greater risk of developing abnormal lipid levels on isotretinoin. This may promote tighter monitoring of lipid levels in these patients being treated with isotretinoin.

The reported side-effects were largely aligned with what is reported in international literature.^[19,21] The most commonly reported side-effect in our study was xerosis. The prevalence in our population was slightly lower than in a large retrospective study, where almost 95% of patients reported the presence of xerosis, and 99% of these tolerated the adverse reaction.^[19] In our study, 19 patients (21%) reported experiencing xerosis, with other patients reporting other mucocutaneous side-effects in line with xerosis such as dry scalp, dry eyes and cheilitis. These side-effects were mostly effectively managed with ocular lubricants and petroleum jelly, barring two patients who had their isotretinoin dose reduced owing to xerosis and dry scalp. There was one patient who had their treatment stopped prematurely at 3 months due to xerosis. Starting treatment at a lower dose of isotretinoin has been shown to effectively improve acne while reducing side-effects, owing to its relatively predictable, controllable and dose-dependent nature.^[19] Managing side-effects, unless severe, with dose titration instead of termination should be considered.^[19,22,23]

Acne vulgaris as a condition has been linked to a high incidence of depression and anxiety due to decreased self-esteem and appearance-related satisfaction.^[21,24] Reports of depression and suicide while taking isotretinoin do exist, although these are uncommon.^[25] Although the causation of depression and suicide due to isotretinoin has not been proven, idiosyncratic reactions cannot be ruled out.^[21] A recent study reported that 10% of participants experienced symptoms such as anxiety, depression, aggression and emotional lability while on isotretinoin.^[26] Our study revealed one patient who had their isotretinoin dose reduced after initiation owing to reported mood swings. Two other patients had reported depressive symptoms after isotretinoin initiation, and therefore did not have their isotretinoin dose increased after initiation as planned. Unfortunately, baseline mental health status was not documented, which may introduce information bias by making it unclear whether the reported symptoms were pre-existing or related to treatment side-effects. Given the retrospective nature of our study, it was also not possible to ascertain whether substance abuse or withdrawal (e.g. from alcohol or illicit drugs) may have played a role. These findings emphasise the need to enquire about all symptoms while patients are taking isotretinoin, including psychiatric side-effects.

Less common, nonetheless noteworthy, side-effects in our study that caused treatment termination included one case of PTC, one case of severe chest pain and one case of exuberant granulation tissue in the healing acne lesions. PTC is a neurological syndrome consisting of increased intracranial pressure, visual disturbances and headaches without a known source such as a stroke, infection, or space-occupying lesion.^[27] The case of PTC is not an isolated or unique case.^[27] Retinoids and tetracyclines have been documented to be among the medications linked to PTC, despite the pathogenesis of PTC not being completely understood.^[28,29] Notably, this patient was not started on prednisone.

Current literature is increasingly challenging the need for routine laboratory monitoring during isotretinoin therapy for healthy

individuals with acne vulgaris, and largely suggesting a more tailored approach. Initially, lipid profiles and liver function tests were assessed weekly and biweekly.^[14] Early work by Barth *et al.*^[13] suggested that only a baseline and single follow-up lipid assessment be done after 4 weeks, stating that although isotretinoin may cause mild, transient increases in serum triglycerides, cholesterol and hepatic enzymes, clinically significant hepatic and lipid derangements were rare. Opel *et al.*'s^[12] article regarding isotretinoin-associated pancreatitis concluded that hypertriglyceride-associated pancreatitis is exceedingly rare and usually idiosyncratic, so could not be prevented. The article suggested that baseline triglycerides and a follow-up test should be done in patients who are 'likely to have elevated triglycerides.'^[12] Subsequent large-scale and systematic studies by Hansen *et al.*^[16] and Lee *et al.*^[4] concurred regarding the early biochemical abnormalities, usually within the first 2 months, also that the abnormalities were mild and seldom influenced management – further supporting reduced monitoring. Hansen *et al.*^[16] further concluded that screening blood tests may not ultimately affect the course of treatment, owing to the infrequency of blood testing abnormalities leading to treatment discontinuation. A more recent article^[3] reviewed >400 studies and concluded that in healthy young individuals, adverse biochemical reactions are extremely rare (<1 in 10 000), unpredictable or symptomatic, and not preventable through routine screening. The article stated that the researchers 'could not identify a single blood test that seemed reasonable to perform routinely, given the rarity of adverse outcomes identified in the literature in healthy young patients, or the rapidity of their clinical development.'^[3] They suggested that selective monitoring should be guided by clinical risk factors.^[3] The most current guideline, published by Reynolds *et al.*,^[1] does, however, still suggest that ALT and triglycerides are checked prior to initiation and at peak isotretinoin dosing, but affirms that laboratory abnormalities in these patients are low, and that there is little evidence to support the benefit of laboratory monitoring to detect adverse events.^[1] Our study largely confirms what has been published in the international literature, and that if patients with potential risk factors are initially identified, and all patients are managed on an individual basis, there may be an opportunity for cost-effective care without compromising patient safety.

Study limitations

This study is unique in that there have been multiple articles published on this topic, but no literature from SA currently contributing to the body of evidence, limiting these articles' generalisability to our setting. The consecutive sampling nature of our study ensures that the study sample is representative of the acne patients attending Tygerberg Hospital. The retrospective nature of the study also allows for the exploration of the uncommon events that have been expanded on. However, the study has some limitations. Since clinical notes were reviewed retrospectively, the analysis relied on the completeness of pre-existing data. Some blood test results were incomplete owing to factors such as electronic gatekeeping, failure to conduct the tests, or patients not returning for follow-up. This introduces concerns around information bias and attrition bias. Furthermore, the data were collected from a single hospital that serves a specific geographical area in SA, and our sample size was relatively small. Investigating other regions within the country and assessing a larger population might yield different results. A larger multi-centre prospective study from SA would render more generalisable results.

Conclusion

There is a growing trend towards tailoring blood tests in acne vulgaris rather than testing routinely. Our study echoes much of the

international literature regarding the infrequency of blood result abnormalities that would cause a change in management in otherwise healthy patients with acne vulgaris on isotretinoin. Although most guidelines currently suggest a baseline and one follow-up test within 2 months, our findings align with Affleck *et al.*^[3] in challenging the need for routine blood monitoring in healthy young individuals without risk factors. Larger studies, particularly from our setting, may provide greater insight into the number of patients who have their management changed by blood tests. In resource-constrained settings such as ours, opportunities to save costs without compromising patient safety should be strongly considered.

Data availability. Data used for this study are available from the authors on request.

Declaration. This study was done in partial fulfilment of JdT's MSc in clinical epidemiology through Stellenbosch University.

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